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BRYN MAWR COLLEGE MONOGRAPHS

REPRINT SERIES, Vol. I., ~~No. I.~~

CONTRIBUTIONS FROM THE BIOLOGICAL LABORATORY

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BRYN MAWR, PENNA., U. S. A.
April, 1901

ANNOUNCEMENT

The Bryn Mawr College Monographs will be issued in two series; the first, the ***Monograph Series***, containing articles that appear here for the first time; the second, the ***Reprint Series***, containing reprints of articles that have appeared in other journals.

It is proposed to publish these monographs in separate numbers at irregular intervals as material is accumulated. The numbers will be combined into volumes of about 500 pages.

The monographs are edited by a committee of the Faculty of Bryn Mawr College, consisting at present of President M. Carey Thomas, ***ex-officio***, Professor Thomas Hunt Morgan (chairman), Professor Charlotte Angas Scott, and Professor Herbert Weir Smyth.

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ERRATA.

Page 564, line 32, for *speads* read *spreads*.
Page 565, line 17, for *persues* read *pursues*.
Page 566, line 25, for *demarkation* read *demarcation*.
Page 568, line 4, for *is* read *its*.
Page 571, line 34, for *breath* read *breadth*.
Page 582, line 29, for *occurence* read *occurrence*.

Regeneration in Bipalium.

By

T. H. Morgan.

With 16 text-figures.

Eingegangen am 18. November 1899.

In the fresh-water triclads the power of regeneration is known to be well developed, but the terrestrial forms have not been examined to any extent in regard to their regenerative power. In the following pages I have described some experiments on one of these terrestrial triclads, Bipalium. Bipalium is not uncommon in green-houses in this country and in Europe, being an exotic land-planarian introduced most probably with foreign plants. Through the kindness of Prof. E. G. Conklin I have obtained a number of individuals of this worm. Also from other sources I have obtained a few more. The first observations that I made during the winter of '97—'98 showed that short pieces of Bipalium would develop into new worms in the course of two or three weeks. During the period of transformation of the old piece into the new worm the piece elongates and becomes narrower, so that the proportions of the new worm are very different from those of the original piece. This elongation is due to changes taking place throughout the entire worm in the old tissues.

The species that I have used is that described by Moseley [1] as Bipalium kewence. It has been found in the forests of Upola, Samoa, by J. J. Lister, and has been imported into many parts of the world with plants from Samoa [2]. It is said that the reproductive

[1] Moseley, H. N., Description of a new Species of Land-Planarian from the Hothouses of Kew Gardens. The Annals and Magazine of Natural History. Vol. I. Fifth Series. 1878.

[2] Gamble, F. W., The Cambridge Natural History. Flatworms and Mesozoa. 1896.

organs of the worms living in green houses never come to maturity, and that the worms reproduce themselves entirely by division.

In green houses Bipalium is to be found under flower-pots, or loose boards lying on damp earth. Some individuals that I have found measured 20 to 25 centimeter in length, and nearly half a centimeter in breadth. The body is somewhat flattened from above-downwards. The head is very flat and extremely mobile. It is light brown in color, and around its margin is a double row of small pigment eye-spots. When the worm is extended the region behind the head — the neck — is somewhat narrower that the rest of the body. A deep black median stripe runs the entire length of the dorsal surface. On each side of this is a broader band more greenish or yellowish in color; and further out at the sides is another longitudinal stripe on each side, that is somewhat darker and narrower than the last. In all there are five of these longitudinal stripes on the upper surface of the body. The presence of these stripes is of assistance in following the changes that take place during regeneration. On the lower surface there is a median whitish stripe and on each side of this a darker stripe.

The histology of Bipalium has been described by Bergendal[1]), but since I have not studied in detail the changes that take place in the tissues themselves during regeneration there is no need of referring at length to this part of Bergendal results. He has found that the worms become more numerous particularly in the autumn, and at this time a large number of pieces were found with new heads in all stages of development. The new head is first indicated by a whitish point (Spitze), that slowly grows larger. At first the stripes of the body are present on the head, but later when the papillae and eyes appear the typical pigmentation of the head is acquired. The position of the new pharynx can be seen, since at the point of its formation the median white band on the lower surface of the body speads out. Bergendal did not observe a spontaneous self-division of the worms, but three times animals, from which a long portion of the anterior end was cut off, pinched off also voluntarily a long piece from the posterior end; and in one case two such pieces were separated. In several instances worms that

[1]) Bergendal, D. '87. Zur Kenntnis der Landplanarien. Zool. Anz. X. 1887. page 218. '92. Über die Vermehrung durch Quertheilung der Bipalium kewence, Moseley. Svenska Vet. Akad. Handl. XXV. 1892.

had divided were found in places where they must have been undisturbed for some time. It is, therefore, not improbable that spontaneous division had taken place, but it is of course also possible that the worms may have been bitten by myriopods, or insects, and in consequence had broken up into pieces.

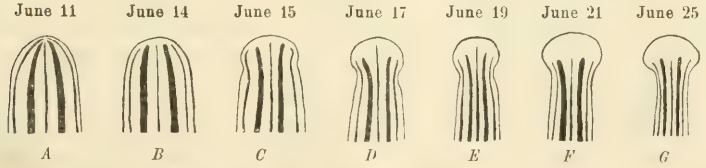
After several trials I have found that the worms or pieces of the worms can be kept in excellent condition as follows. Small flower-pots are filled with earth and the earth kept moist. The pieces of the worm are put on the under surface of the pot where they remain quietly with their ventral surface turned upwards against the pot. The pot is placed on a glass plate, and usually a small wedge of wood is put under one edge. A bell-jar or large tumbler is then inverted over the pot. It is important to have the right amount of moisture present. If too much water is supplied the pieces will macerate; if too little they dry up in a short time.

It is said that Bipalium is carnivorous and that this species persues earthworms engulphing them in its pharynx. »After such a meal which lasts from one to five hours a Bipalium may remain for three months without seeking food.«

The Development of a New Head.

If a worm is cut into several pieces each develops a new head at its anterior end. Soon after cutting the anterior end of the piece bends in, and almost completely covers the exposed part. After twelve hours the end seems to be covered over by the inturned edges and in consequence the five stripes are brought together almost to a point, Fig. I *A*. After three or four days during warm weather

Fig. I.



a whitish tip appears at the anterior end (Fig. I *B*) and the stripes do not now converge as much as at first. A day later the anterior end becomes somewhat rounded and has a flatter wedge-shaped anterior portion, Fig. I *C*. The stripes extend at this time straight forward or nearly so. The anterior end becomes flatter and some-

what rounded, so that the posterior limit of the new head is now indicated. This lies some distance behind the anterior ends of the stripes, and it might seem, therefore, that the substance of the new head is formed almost entirely out of the old tissue. The head now assumes more and more the typical form. The anterior ends of the stripes seem to disappear, Fig. I *D—G*, but whether they are absorbed or whether the head extends forward beyond their limit is difficult to decide. For a long time after the head is fully formed traces of these stripes may still be found.

Meanwhile the piece has decreased very much in width and in height and increased in length as shown in Fig. VII *A*, *D*. The new head is much smaller than the original one, but relatively to the length and width of the new worm it has the typical form and proportions.

At the posterior end of the piece — if the piece was cut from the middle of the body — the exposed tissue is covered in exactly as at the anterior end. The posterior end becomes somewhat pointed and the stripes always extend to near its tip (Fig. VII *A—D*). There is no evidence of an extensive formation of new tissue at the posterior end. Serial sections were made at several stages in the development of the new head. After three days[1]) there appears over the anterior end of the worm, and beneath a distinct layer of ectoderm that now covers the entire anterior end, a certain amount of what seems to be undifferentiated tissue. Its origin is obscure. There is no sharp line of demarkation between the new tissue in the new head and the old tissue behind it. During the following days the new tissue increases in amount and extends forward to form the new head. It was impossible to decide whether this new material is made out of the old tissues that is changed over, as it pushes forward, or whether only new material is added at the free end of the old, and there increases in amount by active growth. Since the pigment stripes that lie in the tissue beneath the ectoderm extend out nearly to the tip of the new head it seems probable that a large part of the material of the new head is made up of some of the cells of the old part that have pushed forward to form the material of the head. Sections show that the new head contains much less material than it might appear to contain when looked at from above;

[1]) The rate of growth depends on the temperature and is much more rapid in the warm summer months that in the autumn at room temperature varying from 68° F. to 72° F.

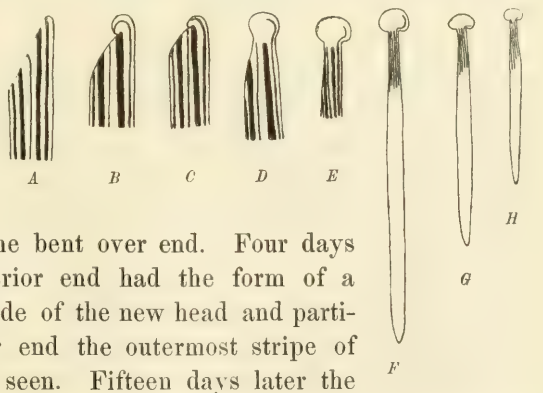
for the new head is formed by a flat plate or wedge pushed forward at the anterior end. The cells of which it is made are at first undifferentiated, but when the head has developed to the stage drawn in Fig. I *F* some of the cells have begun to develop a punctated substance, and have arranged themselves in feather-like groups. When the cut was first made the digestive tract was exposed at the free edge. The new material accumulates in front of the tissue of the digestive tract, and then pushes forward as described above. In later stages the walls of the digestive tract seem to break down in the region just behind the new head.

The following experiments were made to show how the new head forms under different conditions.

An Oblique Anterior End.

A worm[1]) was cut across obliquely as shown in Fig. II *A* (Dec. 11). The pointed end bent over as shown in Fig. II *B* (Dec. 13), so that the outermost stripe of the right side extended entirely around the anterior end. Two weeks later, at an average temperature of about 21° C. the piece appeared as represented in *C*. At this time it looked as though the new head would develop entirely out of the bent over end. Four days later (Dec. 31) the anterior end had the form of a new head. Along one side of the new head and partially around its anterior end the outermost stripe of the right side could be seen. Fifteen days later the anterior end appeared as drawn in *E*, and three later stages are represented in *F*, *G*, *H* (Jan. 27, March 19, May 18).

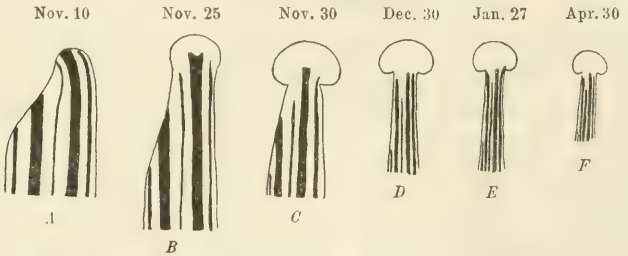
Fig. II.



[1]) This worm had itself developed from a piece and had not grown to its full size when the present experiment was made. The history of the piece is this: on Oct. 24 a large worm was cut into several pieces; two days later the anterior end of one piece was again cut off (Oct. 26); again on Nov. 9 the new head was cut off and another head had developed by Dec. 11 when the piece was cut obliquely as described in the text.

The results show that in this case a new head developed out of only a portion of the exposed anterior end, viz., out of its pointed end. As the head develops the bent-over portion regains to some extent is original position; nevertheless the material for the new head seems to have come in part out of the old tissue of this bent-over portion. It is not improbable that in addition a certain amount of new tissue is added at the left side of the new head. The latter is at first much smaller than the average width of the piece from which it develops, nevertheless, although developing only out of one side of the entire worm the new head is itself bilateral from the beginning; i. e., there is a median plane that will divide it into equal or nearly equal parts, but this plane does not lie in line with the median plane of the rest of the piece.

Fig. III.



The figures show that the head continues to enlarge for a while, and during this time the remainder of the piece is steadily decreasing in breadth and height and increasing in length. These changes lead to the formation of a new worm Fig. II *F*—*H*. In this new worm the head is symmetrically placed in regard to the middle line of the body, but not in regard to the longitudinal stripes of pigment that mark the old planes of symmetry. The last two figures show that the worm decreased in size from January to May. The latter decrease is due, no doubt, to lack of food.

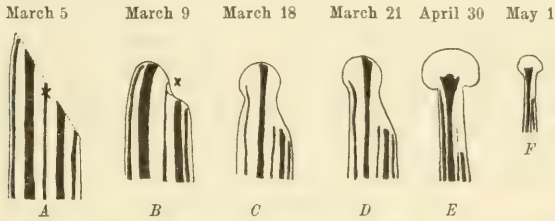
In another experiment the piece was not cut off so obliquely, and the pointed end did not bend over. The series drawn in Fig. III *A*—*F* (Nov. 10—Apr. 30), shows that in this case also a new head develops out of only a part of the exposed end. The new head increases in size as the rest of the piece decreases in width and height and increases in length. In the later stages the head is symmetrical placed at the end of the worm as in the preceding case.

In a third experiment (March 5—May 18) the piece was not only cut off obliquely but afterwards a small portion of the central black

stripe near the anterior edge was burnt with a hot needle as shown by the x in Fig. IV A. During the following days the pointed end shortened somewhat and the region of injury was difficult to see. Therefore the same place was again (after four days) burnt as shown in B. In this series also the head developed out of only the pointed side of the anterior end. As shown in C the inner stripe of the left side occupies the right side of the new head, later (D and E) it lay more in the middle of the new head. As the region behind the head increased in length the anterior end of the middle stripe as well as the ends of the inner and the outer lateral stripes of the right side were left some distance behind the new head as shown in the figures C—F.

These experiments were undertaken, as I have said, with the hope of finding out whether the new head developed in the old

Fig. IV.



tissue, or whether it came from new material. If the head developed out of the old tissue and out of the entire anterior end we should expect to find it at first unsymmetrical. The results show that the regeneration of a new head from a piece with an oblique anterior end takes place in such a way that only at one side of the anterior exposed edge a new head develops and the rest of the exposed side is transformed into the neck-region.

These results led me to undertake a new series of experiments in which I hoped to be able to trace more accurately the changes that take place during the formation of the new head. By means of a hot needle I marked at definite points the upper surface of the piece after its removal.

Marking Definite Points on the Surface.

If with a red hot needle a point of the surface be touched the tissue will be killed in the immediate vicinity of the injured point and a landmark left that can be seen for some time, especially if

one of the stripes of pigment be involved. The anterior end of the middle stripe was destroyed with a hot needle as shown in Fig. V *A* (May 17). Ten days later the anterior end had pushed forward as shown in *B*, and indications of a new head were present. It will be noticed that the lateral stripes of the side have bent in towards each other as they extend forward, while the anterior end of the middle stripe has been left behind. Three days later a new head appeared at the anterior end, *C*. The piece had elongated and become narrower. Six days later, *D*, the new worm was quite well formed, and it was seen that the anterior end of the middle stripe is a long distance behind the anterior end of the worm. I interpret this result to mean that the injury to the anterior end of the middle stripe has prevented its extension forward at the time when the other bands move forward into the region of the new head and neck. The extension forward of pigment bands does not necessarily mean that new tissue is not also formed over the anterior end. The elongation of the outer pigment stripes indicates that some of the old tissue is undergoing changes leading to the formation of a neck-region at the anterior end.

Fig. V.

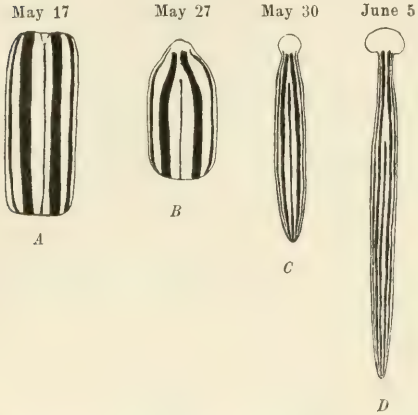
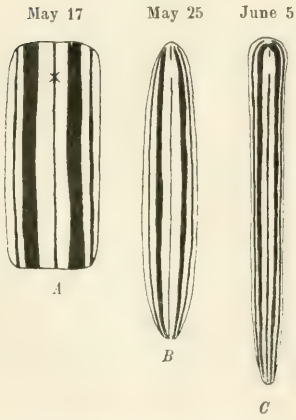


Fig. VI.

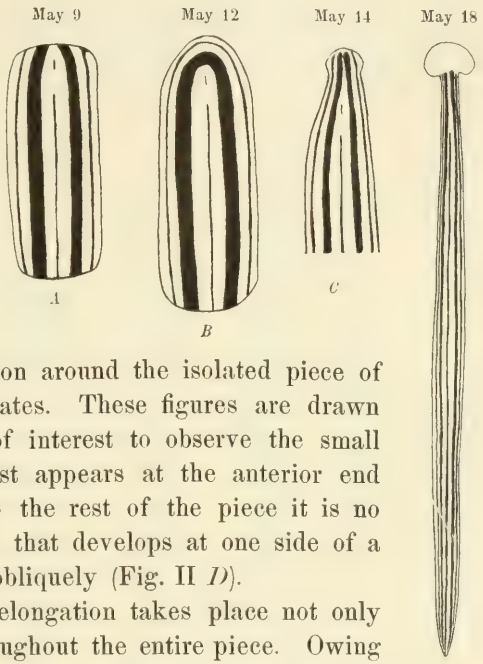


In another experiment the point injured was a few millimeters behind the anterior end Fig. VI *A* (May 17). In this piece the anterior end closed in, but failed to spread out later, and it is of interest to find that no new head developed. The later stages *B*, *C*, show that the outer stripes extend forward around the anterior end of the middle stripe and meet. The anterior end is slightly larger in diameter than is the piece elsewhere, and although the piece was kept until June 5, in all 19 days no evidence of a new head appeared.

The result is important since it shows that the new head can not form directly out of the old tissue of the anterior end unless at one part there remains a free region at which new material is formed.

In two other experiments two points of the surface were injured — one at the anterior end of the middle stripe and the other at a point a short distance behind the first, as shown in Fig. VII *A*. As in the preceding experiments the elongation takes place in front of the anterior end of the middle stripe, although here the entire region around the isolated piece of the middle stripe also elongates. These figures are drawn strictly to scale and it is of interest to observe the small size of the head that at first appears at the anterior end of the piece. In relation to the rest of the piece it is no larger in *C* than is the head that develops at one side of a piece that has been cut off obliquely (Fig. II *D*).

Fig. VII.



The results show that elongation takes place not only at the anterior end, but throughout the entire piece. Owing to lack of material I have been unable to determine by means of injuries at different points whether the rate of elongation is the same throughout the entire piece. Most probably it is nearly so, since the decrease in breadth takes place at an equal rate at all levels.

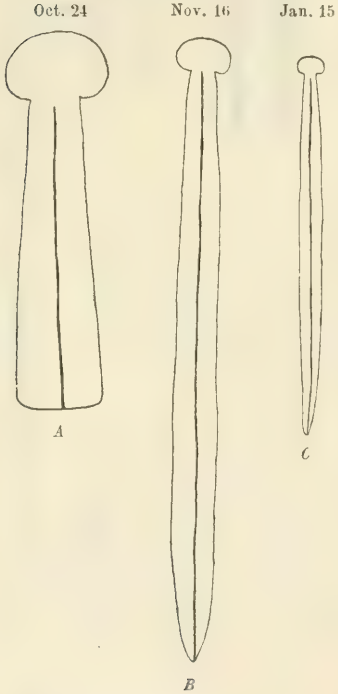
The Transformation of the Head-End into a New Worm.

If a short anterior piece of a worm be cut off, as shown in Fig. VIII *A* it will be changed over, in a comparatively short time, into a new worm. An anterior end was cut off Oct. 24 as shown in Fig. VIII *A*. After twenty-three days it has changed as shown in *B* when it is nearly twice as long as at first, and in breath is as 2 to 3. It will be observed that the head has also decreased proportionately. After nearly three months (Jan. 15) the new worm was smaller, *C*, due most probably to lack of food.

In this piece the head has decreased at the same rate as the body.

If as some of the preceding experiments seem to show the old tissue plays an important rôle in the formation of the new head what will happen if the old head be partially cut off? Will the part that remains make a new head or will the missing part be added at the side of the remaining part? By means of the following experiment I have tried to answer this question.

Fig. VIII.

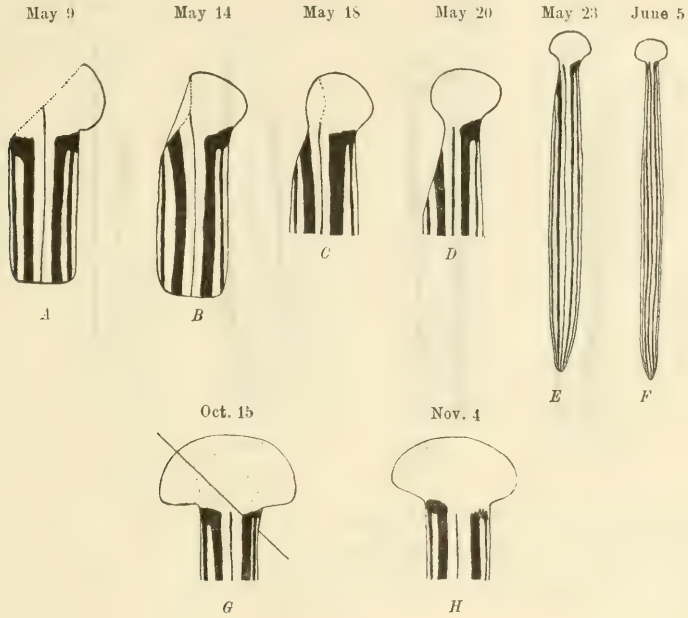


I removed two-thirds of the old head as shown in Fig. IX *A* (May 9). The anterior end was then cut off at the neck — the length of the piece being shown in the figure. The subsequent development of this piece is shown in the series of figures *B*—*F*. The material of the old head is in part worked over into a new smaller head. Also along the cut edge of the head a certain amount of new material is formed, — the material on this side is lighter than that elsewhere, but the amount of this new material is small and less than the part removed. The new head shown in *C* is not half the size of the old head before injury. After twelve days, *D*, the new head is practically formed. Later, *E* and *F* (May 23 and June 1), the new worm appeared to have reached its definitive form, when the new head appeared symmetrically placed at the end of a long body; but the old pigment stripes have not regained a symmetrical position. The new head that had developed fourteen days after the operation, *E*, became smaller during the following thirteen days.

In this experiment the new head did not reach the size of the old one and this was probably because the new worm developed from only a piece of the original entire worm. In order to test this view I cut off (Oct. 15) obliquely the anterior end of an entire worm (17 centimeters in length). The oblique line in Fig. IX *G* shows that

only about $^1/_4$ or $^1/_5$ of the old head remained. The old head measured before the operation 4 mm in breadth and about $2^1/_2$ mm in length. Several days after the operation a number of pieces pinched off from the posterior end of the worm and five small worms developed from these pieces. The worm was correspondingly shortened and measured 12 centimeters in length. New, whitish material soon appeared at the cut end and in 18 days a new head had developed

Fig. IX.



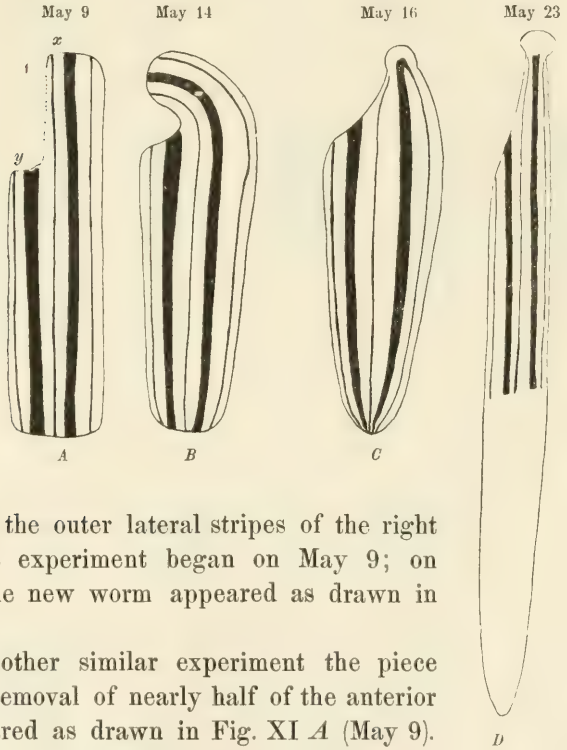
as shown in Fig. IX *H*. The new head was at this time nearly the size of the original one (it measured $3^1/_2$ by $2^1/_2$ mm). Somewhat less than half, but more than $^1/_3$ of the new head is formed of the darker older part; the rest of the head is made up of new whitish material. The result shows that the piece of the old head has increased in size during the development of the new head, and also that new material is formed at the side.

One Half of the Anterior End of a Piece Removed.

In another series of experiments a half was cut out of one side of a piece as shown in Fig. X *A*. Under these circumstances an anterior part is exposed at two levels nearly equal in extent, *x* and *y*.

The possibility of a head forming at each level would seem to be present, and if this should happen I hoped to see what proportions the new piece would assume, — whether for one or for two heads. The series of figures *A*—*D* show that only a single head developed, and at the most anterior end. Figure *C* shows that the new head began as a very small area into which extends the middle, the

Fig. X.



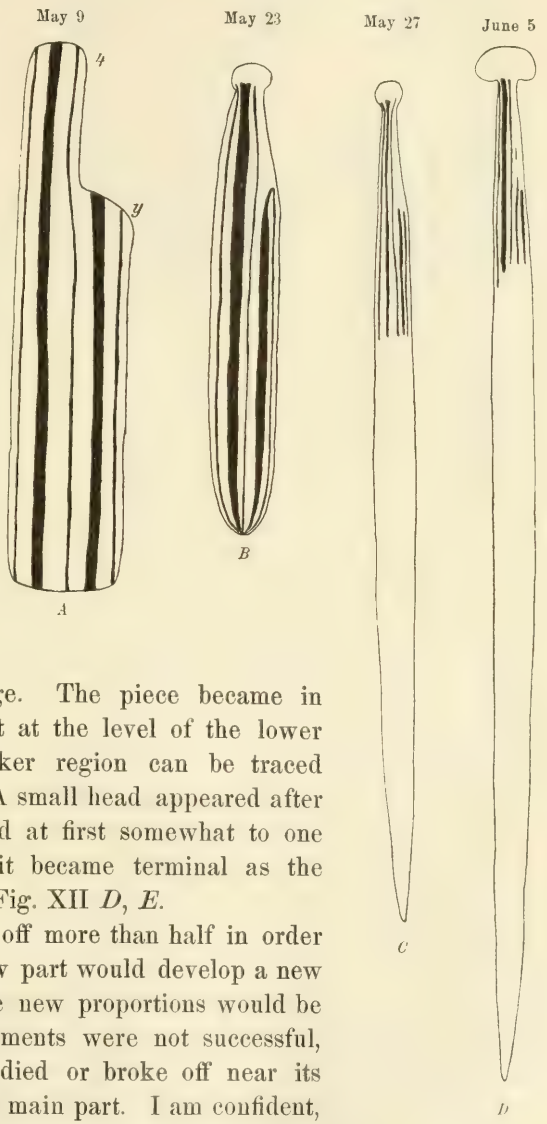
inner and the outer lateral stripes of the right side. The experiment began on May 9; on May 23 the new worm appeared as drawn in Fig. *D*.

In another similar experiment the piece after the removal of nearly half of the anterior end appeared as drawn in Fig. XI *A* (May 9). In this case also a head developed only at the anterior end as seen in *B* (May 23). The subsequent development of the piece is represented by *C* (May 27) and *D* (June 5). The new worm was very long, and judging from the position of the anterior ends of the outer bands of the right side the elongation must have taken place to a large extent from the broader part of the piece, i. e. from the part posterior to the level of the cut at *y*. The results seem to show, therefore, that the greatest elongation has taken place where the piece is broadest. In consequence of this the posterior region reaches the proper

proportion for the anterior neck-region, the latter having developed out of only the left half the body of the old worm.

In a third experiment the part left at one side was a little less than half the width, Fig. XII *A*. At the anterior end of the half-part a little bit of the middle stripe was left, but behind this the knife passed to the left of the middle line. The subsequent history of this piece was not very different from that of the last. In this case the piece had been cut further down leaving a longer half-part. This half contracted very much and partially fused with the lower cut-edge. The piece became in consequence thicker just at the level of the lower edge, *y*, and this thicker region can be traced throughout the series. A small head appeared after fourteen days, *C*, turned at first somewhat to one side, but subsequently it became terminal as the neck-region elongated, Fig. XII *D*, *E*.

Fig. XI.

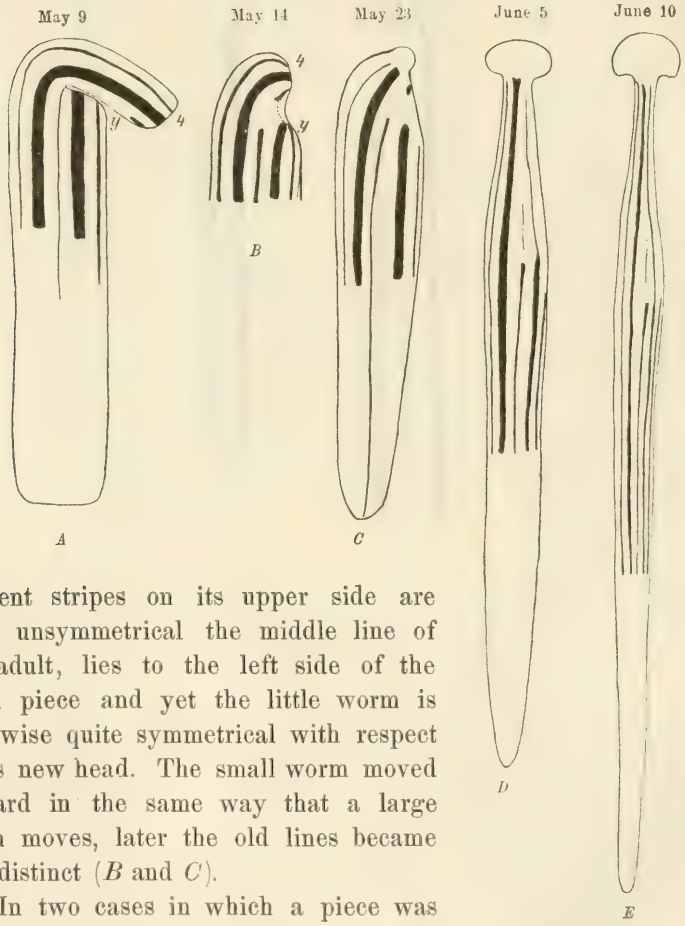


Several times I cut off more than half in order to see if a small narrow part would develop a new head, and if so how the new proportions would be reached, but the experiments were not successful, because the small part died or broke off near its point of union with the main part. I am confident, however, that the experiment could be carried out if one had an abundance of material.

The small pieces removed from the side in the preceding experi-

ments develop in some cases into new worms. In one experiment in which more than half of the side of a piece had been cut off the smaller part broke off later and both it and the piece cut off made small worms. One of these is drawn in Fig. XIII. The

Fig. XII.



pigment stripes on its upper side are quite unsymmetrical the middle line of the adult, lies to the left side of the small piece and yet the little worm is otherwise quite symmetrical with respect to its new head. The small worm moved forward in the same way that a large worm moves, later the old lines became less distinct (*B* and *C*).

In two cases in which a piece was removed from the side Fig. XIV *A* the anterior half-part bent over and fused with the lower part as shown in Fig. XIV *B*. In consequence of this the right side of the half-part now comes to lie, in part, over the anterior end of the piece. The parts fused together so completely that not any of the inner tissue was left exposed. The series of figures, XIV *B*—*E*, shows the subsequent history of this piece. It will be seen

that no new head formed at all, although the piece elongated in the same way as it does when a new head is developing. This result shows that the elongation is not directly connected with the development of a head at the anterior end — a point of some interest. Finally I may mention briefly another attempt to produce in a different way two heads at the anterior end. My object being to

Fig. XIII.

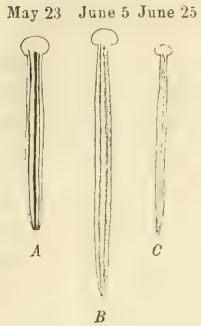
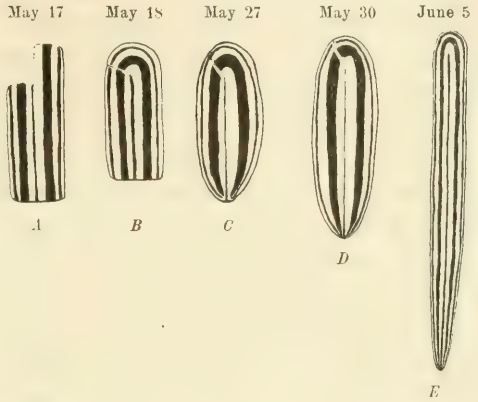


Fig. XIV.



see how the rest of the piece would change its proportions if two, instead of one head were present. After cutting off a piece it was split in two along the middle line at the anterior end. In the course of a few days the two halves had grown together. The end was then split again, and again it closed. At this time the new head had begun to appear. It was split a third time but the two halves came together and a single head developed in spite of the division, three times, of the anterior end.

Grafting.

During the winter of '97—'98 I observed on several occasions that a piece had bent around on itself when it had come to rest, and the two ends happening to come in contact grew together thus making a ring out of the piece. Under these circumstances I noticed that the head did not develop, but at that time I did not succeed in keeping any of the pieces for a sufficiently long time to give a final result. On one occasion I cut in two one of these rings near the point of union, but since from the nature of the case only an anterior end would be exposed at one end of the piece, however, the

ring had been cut, and a posterior end at the other end the experiment could have no important result. The piece died, however, before regeneration began.

The fact that the pieces united so readily suggested that it would be possible to graft pieces together in any desired direction, and after several attempts I have found a method by which this can be done. Two ordinary microscopic slides of the right thickness are laid down near each other, side by side, on a plate of glass, The space between the two slides is just sufficient for the pieces of the worm to lie in. Two short pieces of a worm are then placed between the two slides and their cut ends, — anterior or posterior as the case may be — are brought in contact. Then a small piece of glass is laid over the slides and across the region occupied by the fragments of the worm. The slides etc. are then covered by a bell-jar, and a wet sponge placed inside to keep the atmosphere moist. It is well to keep the bell-jar covered by an opaque cloth since the pieces are restless in the light.

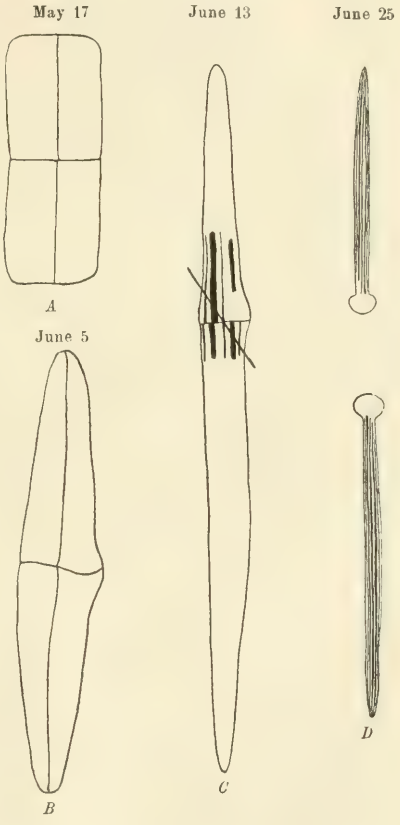
In most cases the pieces of worms that have been brought together crawl apart after a short time, occasionally they remain sticking together. If they separate, as most of them do, they may be brought together again and again until they remain in contact with each other. Even after twenty-four hours I succeeded in uniting two pieces. The principal precautions are to have the space between the slides of exactly the right size, and the amount of moisture around the pieces neither too much nor too little. If too much water collects around the pieces they crawl away. If the under surface of the two slides is slightly dampened they will keep their place better, and supply enough moisture to the pieces.

I have obtained two unions that were permanent, since they held together for nearly thirty days after which time the worms were used for other experiments.

In one case two pieces were united by their anterior ends, Fig. XV *A*. The ends united squarely so that no tissue was exposed. The pieces were kept for twenty-seven days, but during all this time no head developed. The double piece elongated until it was five times as long as at first. On one side of both pieces near the line of union (to the right in *C*) the tissue became whiter and the outer pigment stripes partly disappeared. It looked at first as though one or more heads might appear at the side; but later nothing developed. The experiment shows that the development of a head at the anterior

end is prevented by grafting together the exposed anterior ends. If the new head develops out of the old tissue there is no obvious explanation why it should not do so under the conditions of this experiment. On the other hand it is not difficult to see that a union of this kind might prevent the formation of new tissue in front of the old and thus prevent the formation of a new head.

Fig. XV.

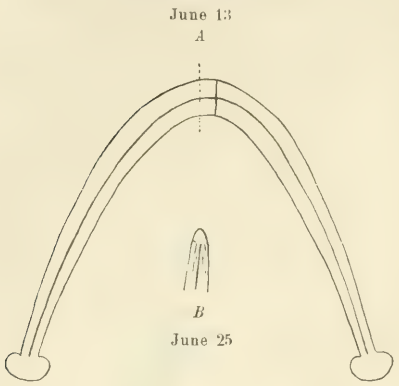


The compound worm was later cut in two by means of an oblique cut (June 13) as shown by the oblique line in Fig. XV *C*. There remained at the most anterior end of each piece a small part belonging to the other piece. Since in the other cases described above, in which a worm had been cut obliquely, a new head developed at the highest part of the exposed surface, I hoped to find out by means of this experiment whether a new head would develop in this case in a similar situation. Should this happen the new head would develop from the posterior edge of the small piece. After the operation the anterior ends contracted somewhat so that it became difficult to distinguish between the two pieces. A new head developed in each case from the upper left hand portion of each, but owing to the contraction of the worm in this region I could not determine positively whether or not the new head developed entirely out of the posterior end of the small component yet such appeared to be the case. In order to determine definitely if this is really the case a larger number of experiments must be carried out.

In another case two pieces failed to unite after several trials. They remained apart over night, but the next morning I succeeded in sticking them together, as I thought, by their anterior ends.

Subsequently a new head developed at each free end (Fig. XVI *A*). There can be, therefore, little doubt that the pieces had been united by their posterior ends since the new heads appeared in the same time that it takes for a head to develop at the anterior end. The pieces elongated and remained together until June 13 i. e. for twenty-seven days.

Fig. XVI.



On June 13 the compound piece was cut apart as shown by the dotted line in Fig. XVI *A*. On one of the two pieces a small part of the other component was left at the posterior end. The exposed edge of the small part was the anterior end. I wished to see if under these circumstances a new head or a tail would develop. As shown in Fig. XVI *B*, the new part that developed resembled a tail and not a head, so that a single worm was formed. The experiment may possibly be interpreted to mean that the small component was too little to develop a new head, and it simply healed over. There are no very marked characteristics that distinguish the tail region of these worms so that the result is not decisive.

Discussion of Results.

As has been stated I began the study of the regeneration of Bipalium in order to determine the method by which pieces of the worm transformed themselves into new worms of smaller size. Owing to the large size of Bipalium it was possible to carry out experiments that would have been difficult or impossible on a smaller form.

The most obvious change that occurs is the decrease in the width and height of the piece and a corresponding increase in length; this change taking place for the most part in the old tissues of the worm. A small amount of new tissue is added to the anterior end and most probably a small amount at the posterior end. The amount of new tissue formed at the anterior end is masked by the moving forward of the longitudinal stripes. These stripes are made up of

pigment cells lying beneath the ectoderm, i. e. in the muscular and connective tissues. It is from these latter tissues that the material of the new head seems to be derived. There appears to be an extensive migration forward of transforming material at the time when the new head is about to appear, so that the new head has the appearance of developing out of the old tissue. That something more than this takes place is shown by those instances in which the anterior end has been closed so that no new tissue can be added in front. Under these conditions no new head appears.

In how far this development of new material at the anterior end is a phenomenon peculiar to that part can not be stated, for it is not improbable that new tissue is made throughout the entire piece. The cells of the body of the planaria are so small that it is extremely difficult to determine the exact origin of new cells by means of sections.

I have pointed out that the new head is at first small, and that it grows rapidly in size soon after its formation. At the same time the rest of the worm is decreasing in two diameters and elongating in the third. The latter change is not, however, correlated with the development of the new head since it takes place also in those cases in which no new head is formed. The new head of a piece does not continue to grow until it has reached the size of the head of the worm from which the piece was taken but stops growing after a certain smaller size has been reached; the size depending upon the size of the piece itself. This is true, of course, only when a piece of the worm has been removed, for when the head has been cut off from the anterior end of a large worm the new head ultimately attains the original size.

It is important to bear in mind that the growth of the new head will take place in a worm that is slowly decreasing in size owing to lack of food. In other words a piece that is slowly starving to death will develop by processes of growth a new head if the old one be removed, and the new head, that increases in size till its proper proportions are reached, is formed out of the starving tissues of the worm.

It is most remarkable that when a piece has an oblique anterior end the new head appears at the pointed tip and, therefore, at one side of the piece. The new head is from the start symmetrical in itself or nearly so although its middle line lies far to one side of the middle line of the old piece. In the course of a few weeks the

piece has decreased in size until the head lies symmetrically at the anterior end of the new worm. If the longitudinal stripes of pigment be examined it will be found that now they are more nearly symmetrical with respect to the new head; this is owing, in large part, to the decrease in size of the piece behind the head so that the unsymmetrical lines appear to be more nearly symmetrical. But an examination of the new worms that develop from pieces from one side of the body (Fig. XIII) will show, that the middle line of the body of the new worm may be formed without regard to the old middle line that is marked by the middle pigment stripe. The old stripes remain on the new worm, but they no longer indicate the new median plane of the new worm. How far these relations might change if the pieces be fed and thus made to grow can not be stated.

In a previous paper[1]) I have drawn attention to a similar process of remodelling or remoulding that takes place in the regeneration of Planaria maculata. I proposed the name morpholaxis for the process. It may be well to indicate how the process of morpholaxis is related to the process of regeneration. Without attempting to discuss here the various definitions of regeneration that have been given I may state that in my opinion it is useful to use the word in as broad a sense as possible and not attempt to narrow its scope as Driesch has proposed to do. If it is necessary to give other names such for instance as heteromorphosis, self-regulation, morpholaxis etc. to certain kinds of regenerative processes it seems to me better to surbordinate these terms to the more general term regeneration. The other words are useful to express the different ways in which regeneration takes place.

The process of morpholaxis is I think, of more general occurence than has been generally supposed. For instance it seems to be the method by which all the protozoa regenerate. It occurs also in Hydra for it seems that in this animal there is little or no development of new material but a remodelling of the part into a new form. In tubularian hydroids a similar process occurs, and also as I have shown in fresh-water planarians.

In these groups, the Protozoa, the Coelenterata, and the Turbellaria the normal growth of the forms takes place throughout the

[1]) Experimental Studies of the Regeneration of Planaria maculata. Archiv f. Entwickelungsmech. VII. '98.

entire body. They seem to increase in size by the addition of new material everywhere in the old parts. The possibility of extensive remodelling would seem to be present in such forms, and during regeneration we see that an extensive process of morpholaxis does take place.

In other groups in which the body is formed by a repetition of similar or nearly similar parts, as for instance the Annelids the Vertebrates, and the Echinoderms[1]) growth takes place in part by the enlargement of each segment and in part by the addition of new segments at the distal end. When a portion of the body of these forms is cut off there are two possible ways in which the development of a new form out of the piece might take place; either by the division of the segments themselves to form the normal number, or by the addition of new segments at one or both ends. The former process is not known to take place in the normal growth of these animals, the latter process does takes place, at least at the posterior end, and we find that during regeneration the lost part is replaced by the addition of new segments at both ends.

I have wished to draw attention to the similarities of normal growth and of growth by regeneration because the comparison shows us, I think, that there is a fundamental agreement between the two phenomena.

Summary.

1. If Bipalium is cut into several pieces each piece makes a new worm. A new head develops at the anterior end by the formation of new tissue in that region. The old longitudinal stripes of pigment extend at first out upon the new head (Fig. I *A*—*E*) producing the effect of a head developing directly out of the old tissue. Serial sections show, however, that new material is first formed in the region of the head but the pigment stripes of the body are also carried out at this time into the new region.

At the posterior end very little new tissue is formed. The elongation of the piece takes place, therefore, mainly in the old tissues. A short broad piece produces a long narrow worm (Fig. VII).

2. If the worm is cut obliquely in two (Figs. II, III, IV) the new head appears not in the middle line, but at one side, and near

[1]) The arms at least of these forms.

the highest anterior edge. The new head is bilateral but its median plane does not correspond to the median plane of the rest of the piece. The old pigment stripes of the body are no longer placed symmetrically with the median line of the new head.

3. If a point on the upper surface be marked by killing the tissues with a hot needle (Figs. V, VII) the elongation of the anterior region of the body can be more definitely observed. There is then shown to be a distinct moving forward of tissue anteriorly.

4. In two cases (Figs. VI, XIV), the anterior end closed, and under these circumstances no new head developed, but the piece as a whole elongated as it does when a head is present. By grafting together two pieces (Fig. XV) by their anterior ends no new heads appear. All these cases show that by the prevention of the formation of new tissue at the anterior end no new head develops.

5. A short anterior end containing the old head (Fig. VIII) develops into a new worm; the old head decreases proportionately with the rest of the piece.

6. If a half or more of the head be cut off at one side (Fig. IX) the remaining part forms a portion of the new head. New tissue also appears at the cut-side.

7. If the end of a piece be cut longitudinally and then one half be cut off as shown in Fig. X a new head develops at the anterior end of the half that remains. The entire piece elongates to form a new worm.

8. The small piece cut off in the last experiment may also develop into a small worm (Fig. XIII).

9. Two pieces were grafted together by their anterior ends (Fig. XV). New heads failed to develop. Later when cut apart obliquely as shown in Fig. XV *C* each piece developed a new head, *D*, from the most anterior region of the cut-surface. It seems probable under these circumstances that the small piece of the other component took part in the development of the new head.

10. Two pieces were grafted together by their posterior ends (Fig. XVI). Each developed a new head at the free end. When cut apart as indicated by the dotted line in the figure (XVI *A*) the shorter piece left on one component failed to develop a new head.

Bryn Mawr College, Bryn Mawr, Penn. U. S. A., Nov. 2 99.

Zusammenfassung.

1. Wenn man ein *Bipalium* in mehrere Stücke zerschneidet, so entsteht aus jedem derselben ein neuer Wurm. Am vorderen Ende entwickelt sich ein neuer Kopf durch die Bildung neuen Gewebes in dieser Gegend. Die alten Pigmentlängsstreifen erstrecken sich von Anfang an auf den neuen Kopf weiter Fig. I *A—E*), was den Eindruck hervorbringt, als entwickelte sich ein Kopf unmittelbar aus dem alten Gewebe. Schnittserien zeigen aber, dass zunächst neues Bildungsmaterial in der Kopfgegend entsteht, während gleichzeitig die Pigmentstreifen des Körpers in die neu sich bildende Region hinein auswachsen.

Am hinteren Ende des Stückes wird nur sehr wenig neues Gewebe gebildet. Die Verlängerung des Stückes findet daher wesentlich mit Hilfe und im Gebiete des alten Gewebes statt, wenn aus einem kurzen breiten Stück wieder ein langer schmaler Wurm entsteht (Fig. VII).

2. Wenn der Wurm schräg entzweigeschnitten wird (Fig. II, III, IV), erscheint der neue Kopf nicht in der Mittellinie, sondern an einer Seite und zwar in der Nähe des äußersten vordersten Punktes des Vorderrandes. Der neue Kopf ist zwar bilateral ausgebildet, aber seine Mittellinie bildet nicht die Fortsetzung derjenigen des ganzen übrigen Stückes. Die ursprünglich vorhandenen Pigmentstreifen des Körpers werden dann auch nicht mehr symmetrisch zur Mittellinie des neuen Kopfes angelegt.

3. Wenn man sich einen Punkt der oberen Körperoberfläche dadurch bezeichnet, dass man mit Hilfe einer heißen Nadel eine kleine lokale Gewebsnekrose hervorbringt (Fig. V, VII), so kann man die Verlängerung der vorderen Körpergegend mit größerer Sicherheit beobachten. Dort bemerkt man dann ein deutliches Vorwärtswandern des Gewebes nach dem vorderen Ende zu.

4. In zwei Fällen (Fig. VI, XIV) schloss sich die Wunde am vorderen Ende und unter diesen Umständen gelangte kein neuer Kopf zur Entwickelung. Es verlängerte sich aber das Stück im Ganzen wie beim Vorhandensein eines neuen Kopfes. Pfropft man zwei Stücke mit den Vorderenden auf einander, so erscheint kein neuer Kopf.

5. Ein kurzes Vorderende, welches den alten Kopf enthält, entwickelt sich wieder zu einem vollständigen neuen Wurm. Der alte Kopf nimmt dabei, seine Verhältnisse denen des übrigen Stückes anpassend, an Größe ab (Fig. VIII).

6. Wenn die Hälfte oder mehr vom Kopf seitlich abgeschnitten wurde (Fig. IX), so nimmt der Rest an der Bildung des neuen Kopfes Theil, indem auch an den Rändern des Schnittes neues Gewebe gebildet wird.

7. Wenn man ein Ende der Länge nach spaltet und dann eine der beiden Hälften entfernt, wie es Fig. X zeigt, so entwickelt sich an der übrig bleibenden Hälfte ein neuer Kopf. Das ganze Stück verlängert sich, um einen neuen Wurm zu bilden.

8. Das im vorigen Versuch abgeschnittene Stück kann sich gleichfalls zu einem kleinen Wurm entwickeln.

9. Bei zwei mit den Vorderenden zusammengefügten Stücken kam keine Kopfneubildung zu Stande. Als dieselben später durch einen schrägen Schnitt wieder getrennt wurden (vgl. Fig. XV *C*), bildete jedes Stück einen neuen Kopf (*D*) im allervordersten Bezirk der Schnittfläche. Es erscheint bei dieser Versuchsanordnung wahrscheinlich, dass ein kleiner Theil des anderen Komponenten sich bei der Regeneration des Kopfes betheiligte.

10. Bei der Verbindung zweier Stücke mit den Hinterenden (Fig. XVI) bildete jedes am freien Ende einen neuen Kopf. Nach Zerschneidung an der durch die punktirte Linie angedeuteten Stelle (Fig. XVI *A*) bildete das kurze an dem einen Komponenten hängenbleibende Stück keinen neuen Kopf.

ERRATA.

Page 71, line 11, for *ower* read *over*.
Page 98, line 13, omit period after parenthesis.
Page 108, line 1, for *relinguish* read *relinquish*.
Page 113, line 11, for *principle* read *principal*.
Page 116, line 13, for *be the* read *may*.

Regeneration in Planarians.

By

T. H. Morgan.

With 31 figures in text.

Eingegangen am 27. April 1900.

The results of my previous study of the process of regeneration in Planarians ('98, 1900) served to clear the way for further work. The main result that I obtained was in relation to the change of shape that the old part undergoes in the production of a new individual. The present account attempts to deal more fully with this subject, that I have proposed to call Morpholaxis. Other problems have also been examined and are here considered.

The experiments described in the following pages were carried out during the winter of '98—'99, during the following summer at Woods Holl, and again during the present winter at Bryn Mawr. The material used included Planaria maculata found at Woods Holl (and also kept in aquaria at Bryn Mawr), two planarians found at Bryn Mawr, one of these quite similar to Planaria maculata and probably the same species, (it has not been used in any experiments described in this paper); the other a larger, sluggish animal Planaria sp. ?.

Incidently I have also used Phagocata gracilis and Dendrocoelum lacteum from Woods Holl, and Bipalium kewense — a tropical land planarian found in green-houses.

The experiments recorded in my previous paper were carried out without feeding the new worms, for I did not then know how readily they will take food in confinement. In many of the experiments described in the present account the planarians were fed from time to time. In my previous paper I pointed out that the worms after

regenerating decreased in size slowly from month to month. This change I correctly ascribed to lack of food. If the new worms are fed every week or oftener they increase in size, and the regenerative changes take place more completely. During the summer of '99 I found that my planarians would eat greedily pieces of the common house-fly that were put into the water. Moreover I found that if the head of the fly containing the red-pigmented eyes was given to the planarians, the red substance when taken into the digestive tract would color that organ, in all its parts, a brilliant red, as though a differential stain had been used for the digestive system. In consequence of this color it is easy to follow from week to week the development of the new digestive tract, and the results have added some points of interest to the work. The experiments in this account are not described in their chronological order, but in accordance with the nature of the experiment.

Regeneration in an antero-posterior direction.

If a planarian is cut into a number of cross-pieces each piece develops new tissue at its anterior and posterior ends, and at the same time there is a corresponding loss in the material of the old part. The new material at the anterior end very early differentiates into a new head, and the new material at the posterior end makes a new tail. The new worm is much too broad for its length, i. e., it lacks the proportions of length to breadth characteristic of the normal worms, either young or old. In the course of a few weeks changes take place in the worm that remodel it into the characteristic form.

If the changes are carefully followed it will be noticed that the new material that is first laid down to form the new head and tail does not increase very much in length, at least not sufficiently to account for the greater part of the entire change in length. The old part on the other hand soon becomes longer than it was at first, and at the same time narrower. This decrease in breadth (and also in height) is due at first to the formation of the new substance of the head and tail, but after these structures have been formed the old part still continues to get narrower, and at the same time to increase in length. At this time the region between the old and the new parts grows lighter indicating that changes are taking place in it that lead to a rapid increase in length, particularly in the anterior

region. Obviously this increase in length also brings about a corresponding decrease in breadth.

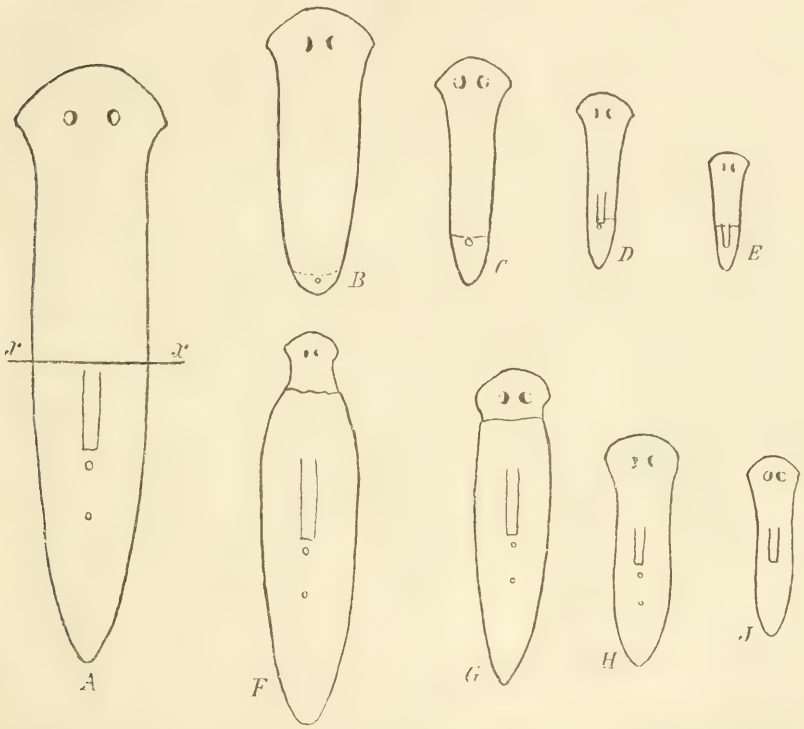
One further point should be recalled. The position of the new pharynx in the cross-piece is dependent upon the part of the body from which the piece has been taken. If the cross-piece is taken from the region in front of the old pharynx, then the new pharynx appears at the posterior edge at the border line between the old and the new parts, i. e., it appears near, or in that region of the piece that lay nearest to the old pharynx. If the cross-piece comes from the region of the old pharynx, then the new pharynx forms in the middle of the piece in the old tissue, and presumably out of some part of the old pharynx, or its surrounding chamber. If the cross-piece comes from behind the old pharynx, the new one comes in either in the anterior part of the old tissue, or else between the old and the new tissue at the anterior cut-edge. There is some difference between different species in this respect. This relation between the position of the old and of the new pharynx is a point of some theoretical interest especially in regard to the question of the so-called polarity of the regenerating piece, but I shall defer a discussion of this question until later.

In order to study the morpholactic changes that take place in the formation of a new worm a number of planarians were cut in two just in front of the pharynx, or through the pharynx; or in front of the genital-pore that lies behind the pharynx. These structures give definite landmarks by means of which the changes taking place can be more definitively studied.

A worm (Planaria sp.?) was cut in two as shown in Fig. 1 *A*, through the region in front of the pharynx as indicated by the line *x*—*x*. The history of the posterior piece, *F*—*J*, is as follows. Seven days after the operation a new head was present. At this time the region of old material in front of the pharynx had noticeably increased in length, Fig. 1 *F*, and as a result the new head was carried further forward. After 18 days the new worm had decreased somewhat in length, Fig. 1 *G*, owing to lack of food, and was somewhat more than half its former breadth. The latter change being due for the greater part to its previous increase in length, and also in part to the lack of food. After 21 days, Fig. 1 *H*, although much smaller the worm had approximately attained its normal proportions. It continued to decrease in size as shown, after 40 days, in Fig. 1 *J*.

The anterior half of the same worm formed at first a small amount of tissue at its posterior end Fig. 1 *B*, and a new, very short pharynx appeared in the new part. This new part at the posterior end increased a little in length, Fig. 1 *C*; but after two months it was only as large as seen in Fig. 1 *D*. The worm continued to decrease in size, until 40 days later it measured only $^2/_5$ its former length, and $^1/_4$ its former width Fig. 1 *E*. The pharynx does not

Fig. 1.



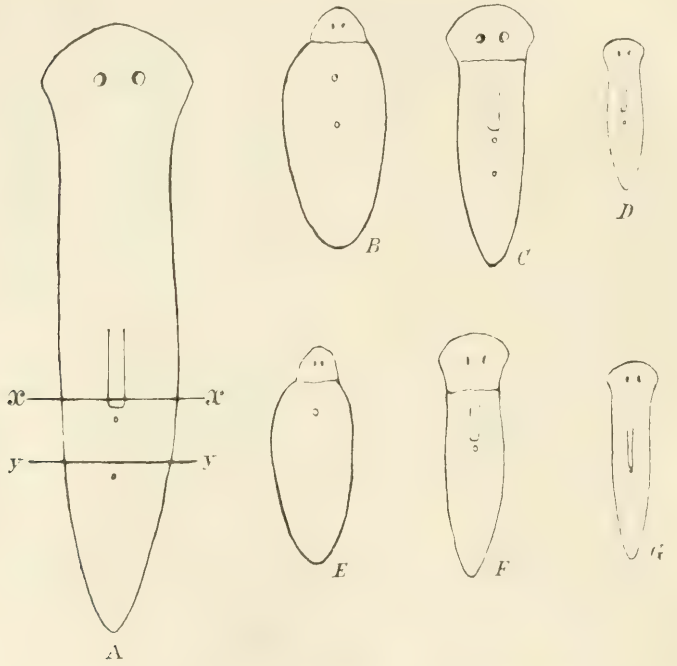
appear as much displaced as at first, but this change is due more to the decrease in the whole piece as a result of starvation of the tissues, than to a readjustment of the proportions of the piece; for, as shown by the figures the pharynx is still as near as before to the posterior end. This means, however, either that the region behind the pharynx decreases proportionately less than the rest of the worm, or that its growth keeps pace with its decrease — if in fact both processes could possibly go on at the same time.

This decrease in size owing to lack of food is similar to that

I described in my former paper as taking place in Planaria maculata. I shall describe a little later how regenerating worms of the latter species behave if fed.

In the next experiment a worm (Planaria sp.?) was cut in two through the pharynx itself, Fig. 2 *A*, *x*—*x*. The posterior end of the pharynx fell out of the posterior piece. The new head that appeared lay just in front of the pharyngeal pore, Fig. 2 *B*. During the next two weeks (the worm was not feed) the region of old tissue

Fig. 2.



in front of the pore, and behind the new head, increased in length, Fig. 2 *C*, as did also the new head. The worm became narrower as it increased in length, and after a time assumed nearly the normal proportions of the body, Fig. 2 *D*.

The anterior piece added a small amount of new material to its posterior end, and a new pharyngeal pore formed just within the old part. The piece decreased in breadth, and also in length during the following months (without food), but the pharynx still remained too near to the posterior end. There is, as these experiments show, a very noticeable difference in the regeneration of the posterior as

compared with the anterior end. After the formation of a new head and tail the region behind the new head increases rapidly in length, before the changes due to the starvation of the pieces begin to appear; the decrease in the breadth of the piece being due directly to the loss of material to the anterior region. In an anterior piece on the contrary, although the new tail is quickly formed, there is a noticeable stand-still after this, and the new pharynx, although it grows in length, yet remains almost as near the posterior end as at first. Owing to the decrease in size of the whole worm, due to starvation, the pharynx later appears nearer to its normal position in the worm. If the piece is fed, however, the posterior region grows, as described below, to its normal length.

In a third experiment the worm was cut in two between the pharyngeal pore and the genital pore, Fig. 2 *A*, *y*—*y*. A new head was present, after nine days, on the posterior piece, Fig. 2 *E*. As the head increased in size the region in front of the genital pore increased in length. Indications of the formation of a new pharynx in the region in front of the genital pore, and near the edge of the new head, were plainly seen. During the following ten days the new head grew larger, and the intermediate region between the pore and the head increased in length, Fig. 2 *F*. After another ten days the piece had reached the normal proportions, Fig. 2 *G*. This does not mean, of course, that as much new material appeared in front of the pharynx as was present in the original worm, for the increase in length has been at the expense of the region behind the pore, that has correspondingly lost in length and breadth. The new worm is in fact not very much longer than the original piece, for what it gains in front it loses behind. The difference in breadth between the old piece and the new worm would supply enough material to make a certain increase in length possible.

The anterior piece formed a small amount of new material at its posterior end, and a new genital pore appeared just at the border between the old and new parts.

These three experiments are typical of what takes place under the special conditions of each experiment. In the main all three experiments give the same results; the main points being the early formation of a new head and tail, followed in the posterior pieces by a rapid increase in length of the old tissue in the region behind the head, and in the anterior pieces by a noticeable absence of a similar rapid increase in front of the new tail.

In the third experiment the new pharynx developed in front of the genital pore, and can, therefore, only appear after the intermediate region has increased somewhat in amount. It develops much nearer the head than the position of the pharynx in the normal worm. Does the genital pore act, therefore, as a barrier preventing the formation of the pharynx at a more posterior point? In my experiments with Planaria maculata I had found when pieces were cut off in the region of the genital pore that the new pharynx came in promptly in the old tissue some distance from the anterior end, and, therefore, nearer to its definitive position. In order to re-examine this problem I made two new series of experiments on Planaria sp. ?. Three worms were cut in two between the pharyngeal and genital pores as in the last experiment. Four other worms were cut in two just behind the genital pore. Six days later heads were present on all the pieces, and in most of them a pharynx was in process of formation. In the pieces with the old genital pore the new pharynx lay in front of the pore in the intermediate region. In the pieces cut off behind the pore the pharynx lay very far forward just at the edge of the new tissue of the head. It had developed either out of the new tissue, or out of the intermediate tissue, that must also have included much new material as indicated by its lighter color. The results show, that, in this species, the pharynx develops far forward in posterior pieces, without regard to whether the piece has been cut off in front of, or behind the genital pore.

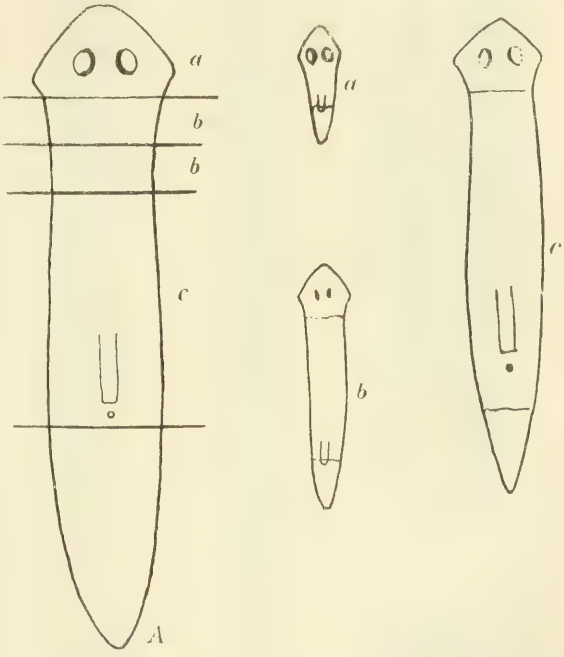
This result led me to re-examine the regeneration of posterior pieces of P. maculata to see if possibly I had been mistaken in stating that the new pharynx developed in the old tissue in this form. Six worms (P. maculata) were cut in two between the pharyngeal and genital pores at different levels between those points (July 2). The worms were exceptionally large; the posterior pieces measuring 4×2, $3\frac{1}{2} \times 2$, 5×2, $4 \times 1\frac{9}{10}$, 6×2, 6×-2 mm. Twelve days later new heads were present and the pieces had narrowed ($3\frac{1}{2} \times \frac{6}{7}$, $6\frac{1}{2} \times 1+$, $6 \times 1\frac{1}{4}$ etc.), and in each an opaque region behind the old genital pore, but anterior to the middle of the piece, indicated the formation of a new pharynx. Later the genital pore disappeared. The results show that in this species the presence of the old genital pore does not determine that the new pharynx shall appear in front of the old pore.

In another experiment (July 6) the posterior ends of six worms were cut off just behind the genital pore. Seven days later a new

head was present, and a new pharynx had appeared. The new pharynx was well within the old part, and lay a little anterior to the middle of the piece, i. e., in the same relative position in these pieces as in the last ones.

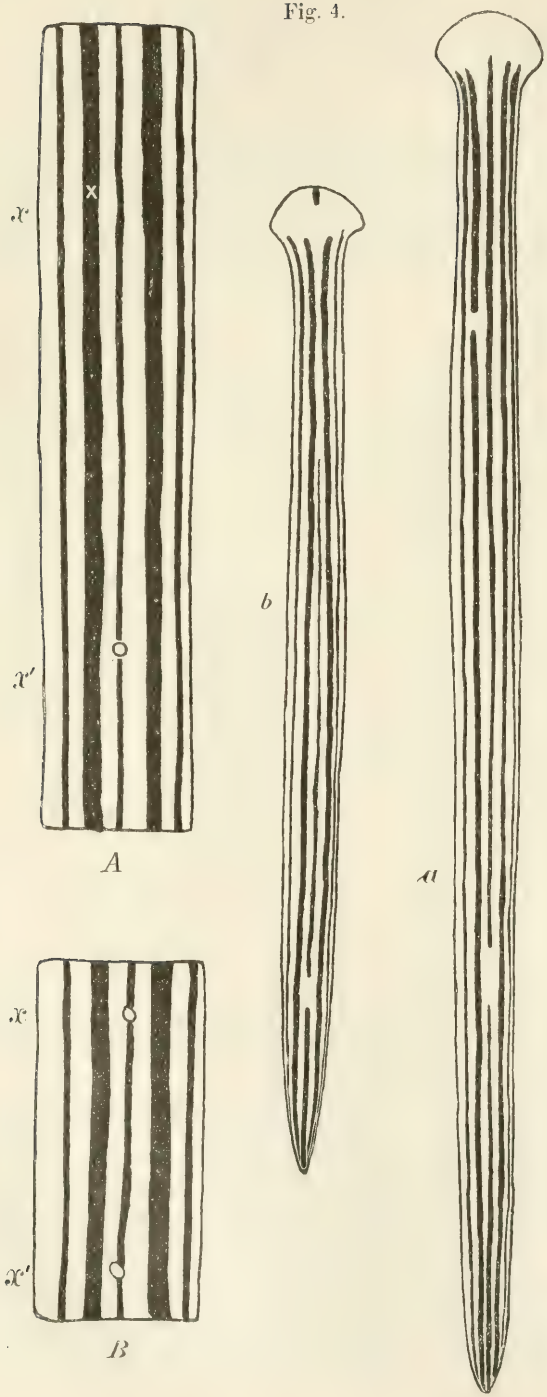
In order to see if the presence of the genital pore delayed the development of the new pharynx the tails of several worms were cut off (July 31) some just in front of, and some just behind the genital pore. On the fifth day after the operation some of the pieces showed evidence of the formation of a new pharynx, but when food was placed near the pieces they were not attracted by it. They gave no response on the day following, only one piece stopped for a short time near the food. On the next day some of the pieces of each kind took food into the digestive tract through the new pharynx. At the same time a third lot of pieces were cut off just in front of the pharyngeal pore. The new pharynx appeared in them at the same time as in the preceding cases.

Fig. 3.



In connection with the problem of morpholaxis the following experiments with cross-pieces are, I think, instructive. Short and long cross-pieces were cut from the body of the same worm. Fig. 3 *a*, *b*, *c*, in order to compare the relative decrease in breadth in pieces of different lengths. When the typical form was reached the worms from the smaller piece were of course smaller, and the old part had decreased more in breadth. The difference in the development of the two kinds of pieces shows most strikingly that the old part not

Fig. 4.



only is changed over into the new worm, but that the change goes on until the normal proportions have been reached. A short broad piece, Fig. 3 *A*, *b*, after forming a new head and tail is much too broad for its length. The piece continues to elongate until the typical ratio of length to breadth has been reached, when equilibrium is established. In order to gain the typical proportions a short piece must lose more in breadth than a longer piece. The change involves both an increase in length, and a decrease in width. To state that the former leads to the latter may put the question in a wrong way. The two changes are so intimately connected that I see no way, at present, of showing that the one is the cause and the other the effect, or that both changes are active ones.

The change may be thought of, either as due to the growth of the new tissue at

the ends at the expense of the food-substances in the old part, or as due to an actual migration of the old tissues forward and backwards. There can be, I think, little doubt that an active migration of old tissue brings about the transformation. That this is the case is best shown by the changes that take place in Bipalium. I have recently made a few more new experiments with this species that clearly show that, in the old part itself, an elongation takes place. A short cross-piece was cut off Fig. 4 *A*, and at two points on the surface, at x and x'. The pigment bands were injured by a hot needle. The distance between the two points was measured as well as their distance from the two ends. No change took place in the relation of these points of injury to each other, or to the ends, until the piece began to get narrower. The greatest increase in length was between the anterior point and the front end, where a very rapid elongation took place, Fig. 4 *a*, but the middle region of the worm also grew longer showing that the old middle part is involved as well as the region nearer the ends.

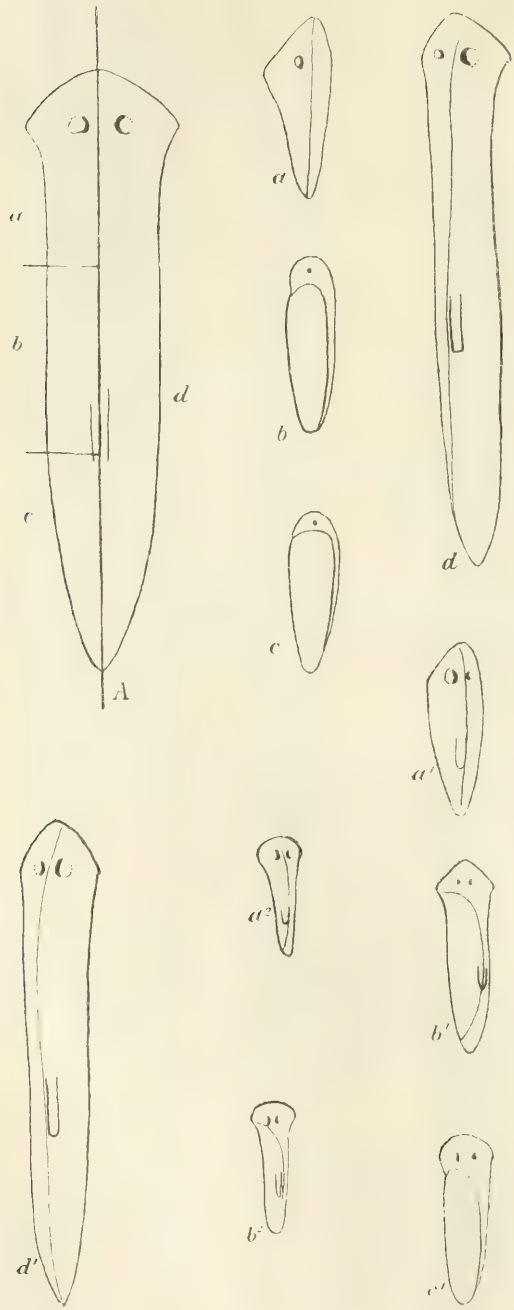
In one experiment a small bit of the middle band was left isolated at the anterior end, Fig. 4 *B*. The bit of pigment came to lie ultimately at the anterior end of the new head, Fig. 4 *b*. This means that all of the old material of which the pigmented piece is composed was carried forward when the material grew out in that direction.

Before leaving this account of the behavior of cross-pieces I wish to record that the closing-in of cross-pieces at the anterior end without the formation of a new head, that sometimes occurs, takes place most often when the anterior cut passes through the pharynx. This region possesses the power of producing a new head, and some of the cross-pieces from this region do make a new head, but a much larger percentage of closed anterior ends results from cross-pieces through this region, than elsewhere. The presence of the large pharyngeal cavity most probably accounts for the difference in closing-in of pieces from this part of the worm.

Lateral Regeneration.

The fresh-water planarians possess to a remarkable degree the power of regenerating laterally. If a worm is cut in two along the middle line of the body each half will make a new worm. The pharynx is usually cut in two in the operation, and hangs out to

Fig. 5.



one side. The greater part sloughs off in a short time, and the region becomes covered over by new tissue. The new material accumulates rapidly along the cut-side until it is about one-third the breadth of the old part. A new pharynx appears at the level of the old one, and just between the old and the new parts — rather more in the old tissue. Its position is at first unsymmetrical. The worm as a whole appears to be bilaterally symmetrical in outline, but the excentric position of the pharynx shows that the piece is, in its internal structure, unsymmetrical. If the worm is fed at this stage the development of new tissue continues, Fig. 6 *d'*, until a symmetrical worm is formed. If the worm is not fed the new part increases very slowly and its increase in width soon becomes obscured by the gradual decrease in the piece as a whole. Fig. 5 *d*, *d'*.

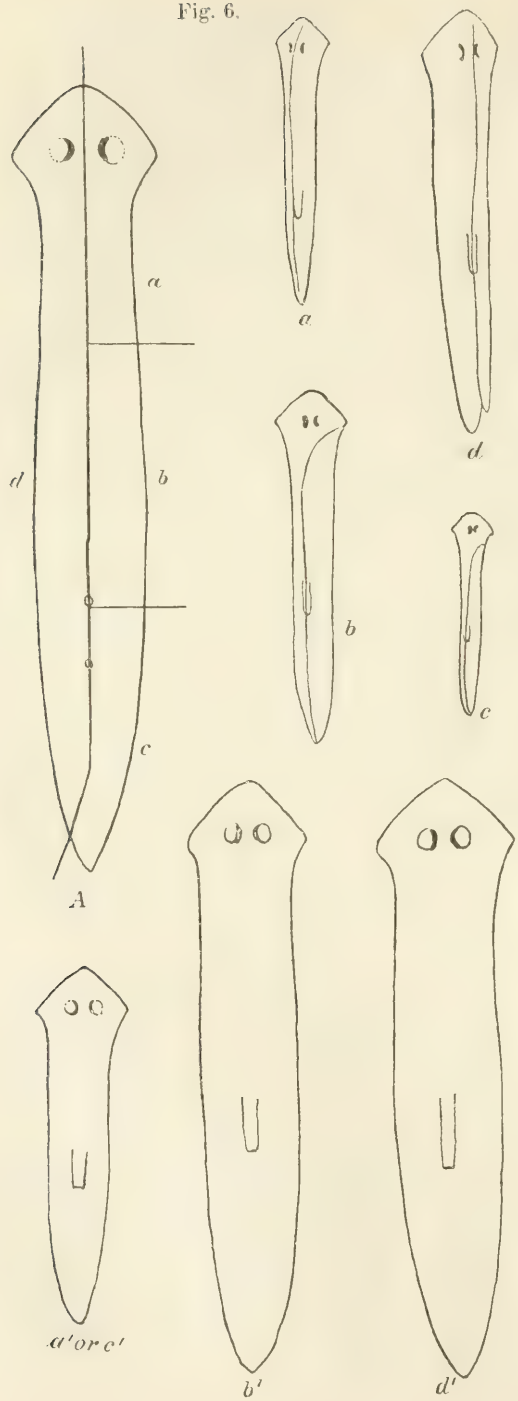
As the new side increases at first at the expense of the old the pharynx will come to lie more and more in the middle line. By means of transportation of material across the piece to form the new side the symmetry may be regained; the new worm being only about as wide as the original piece from which it developed. Measurements show — unfortunately I did not make many of them — that the half-piece shortens at the same time that the new side is growing in width, Fig. 5 *d'*, so that theoretically the piece would become more than the width of the original half, but the gain might be compensated for by the general decrease owing to lack of food during the period of regeneration. The measurements indicate that the new worm is about the same width as the original piece.

If, however, the piece is fed from time to time the new part increases more rapidly, and the old part loses less, Fig. 6 *d*. The new worms soon attain the normal size, Fig. 6 *d'*. It would be interesting to know if the actual migration of material (either substances or cells) from the old to the new side is lessened or prevented by an abundance of food; the increase in the new tissue taking place only by the more rapid growth of that first formed. It would be very difficult, I think, to settle this point.

In the anterior end along the line between the old and the new parts small branches grow out from the old digestive tract into the new part, and a long posterior branch extends backwards on the new side of the pharynx, and posterior to the latter. The new branches are, when first formed, smaller than those in the old part, and it takes a long time for them to become as large as those in the old part. In the course of a month or two the branches of the digestive tract may become alike on the two sides.

In several experiments one of the halves was divided transversely into three pieces and the regeneration of the pieces compared with that of the entire half, Fig. 5 *a*, *b*, *c*. The piece *a* completed the head at the side, Fig. 5 *a*, and the new pharynx appeared near the posterior end at the side between the old and new parts; the pieces *b* and *c* produced a new head at the anterior end, and a new pharynx near the middle of the piece at the side between old and new parts. The amount of new material at the side of the smaller pieces was less in amount than that in the long undivided half-piece. In two of the smaller pieces, *b* and *c*, a large amount of the new material is used in making a new head at the anterior end. In an experiment in which the pieces were fed they

Fig. 6.



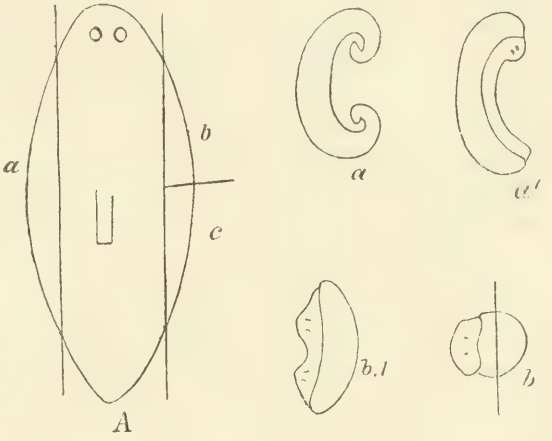
all formed after a time full-sized symmetrical worms, Fig. 6 *a'*, *b'*, *c'*.

In my former paper I described the development of narrow pieces from the side of the worm. Pieces of this sort are much narrower than the half-pieces described above, and contain often much less than half the width of the worm. It has been shown that such pieces produce a new head at the side near the anterior end of the piece, and while some of these pieces form new material at the side behind the head and become typical worms, Fig. 7 *a*, others use up all the material at the side to form one (or two) heads that remain at right angles to the original long axis of the piece, Fig. 7 *b*.

The operation of cutting off these pieces is most easily carried out when the worm is contracted or at rest. The planarian assumes at this time an oval shape. A piece cut off at the side under these conditions is not

cut off strictly in a longitudinal direction, Fig. 7 *A*, *a*; for when the piece stretches out it is thicker in the middle than at the ends, and the cut-edge is a part of the circumference of a large circle. Soon after removal the ends of the piece bend in as shown in Fig. 7 *a*. New tissue appears along the side, and if the piece is large a new head appears at the anterior end turned towards the (original) inner side of the piece, Fig. 7 *a'*. Posterior to the head new tissue appears along the edge to form a new side, and a pharynx develops at the border line between the old and the new tissue. The digestive tract is in many cases a very irregular branched structure, but after a time it changes over into the typical form of that organ. Lateral pieces of this sort, if fed may become full-sized typical worms.

Fig. 7.



If the pieces from the side are small the »right-angled forms«, Fig. 7 *b*, described in my former paper are often formed. In order to produce these forms I generally cut a long narrow strip from the side, and then cut this strip into several pieces by cross-cuts. The piece that comes from the anterior end of the strip, Fig. 7 *A*, *b*, will have its anterior end pointed, and the new tissue can form only along the side. It seemed to me that the right-angled forms might come from these pieces, and the long forms from the others (Fig. 7 *c*), having an exposed anterior cut-edge. I tried, in order to test this view, cutting off pieces from the side, and then dividing each into an anterior and a posterior half, Fig. 7 *A*, *b*, *c*. The more anterior piece has at its anterior end a sharp point, while the posterior piece has an anterior cut-edge. Fifty pieces were cut off in this way. Five days later (July 30) forty were alive. Nearly all had heads at right angles to the side and two had two such heads. Only a few in each lot had a head turned forward. The experiment

showed that there were not produced more right-angled forms in the one series than in the other. Most of the pieces died during the two following weeks especially the right-angled forms. The longitudinal pieces formed long very narrow worms that after feeding several times became larger and symmetrical.

The same experiment was repeated with eight anterior and ten posterior pieces. From the former two right-angled and three longitudinal worms developed; and from the latter two longitudinal worms. After twelve days I tried the experiment of cutting off a portion of the old part, in two of the right-angled forms, Fig. 7 *b*, in order to see if with a free edge the pieces would elongate in that direction and form a complete worm. The cut edge healed and a tail was not formed. These right-angled forms do not, as I have pointed out, produce a new pharynx. This may be due to the fact that in the old part the tissues are arranged at right-angles to those in the new part. I carried out the last experiment in order to see, if, by the removal of a part of the old tissue, the part that remains might under the influence of the new head produce a pharynx and a tail in place of the side piece removed, but, as has been shown, this does not happen. If the new head were entirely cut off from the old part it might produce a new tail and a new pharynx, but the pieces are so small that in the few cases in which I have tried this experiment the head died without regenerating.

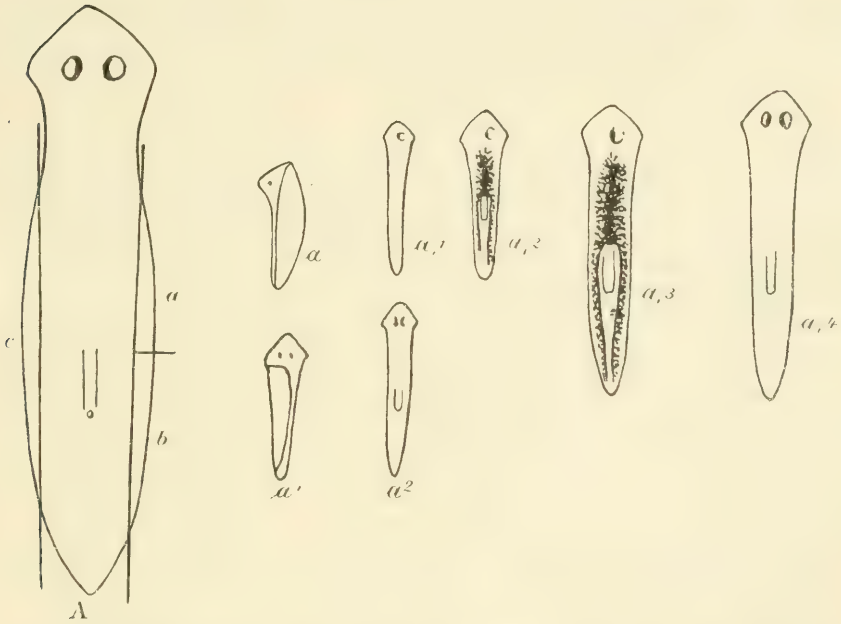
The experiments made with these pieces from the side have thrown a certain light upon the interpretation of the right-angled worms. They develop most often from very small pieces, and as we have seen, without regard to the shape of the piece. The old tissue can give rise in a small piece to only a limited amount of new material. If we assume, and such appears to be the case, that a certain amount of new material is necessary to form a new head, i. e., below a certain amount a head can not form, it follows, that, if a new head is to appear at all on these small pieces, it will use up in its formation all the new material of the cut-side; especially as in these short pieces the new material is continuous and limited to a small area. The size of the new head, in the small pieces, is out of all proportion to the size of the pieces themselves, i. e., the new head is proportionally far larger than the new head on a larger piece.

We should not overlook, however, that this hypothesis assumes there is a stronger tendency to form a head than to form a side

without forming a head; and it also fails to explain how two heads are so often produced if the pieces cut from the side represent pieces long in proportion to their width.

If long, narrow pieces are cut from the side they often form very long narrow worms. Sometimes pieces of this sort develop only a single eye in the new part. In an experiment begun Oct. 1 seven long pieces were cut from the sides of the body of several planarians. By Oct. 13 one had developed a head at the side of the anterior

Fig. 8.



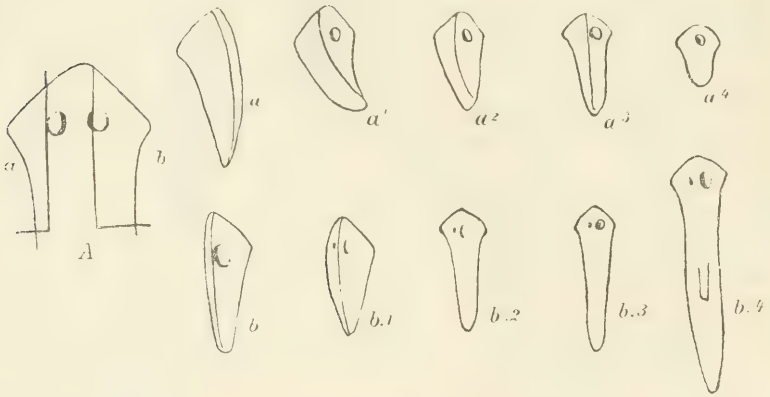
end, one had formed two heads at the side and two had not yet formed heads; the rest were dead. By Oct. 18 there were two longitudinal worms and two right-angled forms with two heads each. By Nov. 5 there were two longitudinal forms, and one two headed right-angled worm. By Nov. 20 the two former (having been fed) had become symmetrical; and by Dec. 22 one piece had grown almost to the normal size, the other was somewhat smaller.

In another experiment carried on at the same time the long pieces, cut off as in the preceding experiment, were cut in two in the middle by a cross-cut. The history of one of these pieces that produced at first only a single eye is as follows. Cut off on Oct. 1

it had regenerated a new head (at the anterior edge) with one eye by Oct. 13, Fig. 8 *a*. By Nov. 5 the piece had changed into a long narrow worm with only one eye — the right one — in the head, Fig. 8 *a'*. It still had only one eye on Dec. 14, Fig. 8 a^3, and had grown larger as a result of feeding. On March 2 another very small, left eye had appeared, Fig. 8 a^4. The result is interesting as showing that a missing eye may form in a new head five months after the new head developed.

It seemed to me a point of some theoretical interest in connection with the lateral regeneration of these small side-pieces to find out how a piece of the head would regenerate, cut off to one side of the eye as shown in Fig. 9 *A*, *a*. A piece of this sort has a

Fig. 9.



definite structure as a part of the old head. It can give rise to only a limited amount of new material at the side — much less than what would be necessary to complete itself on the right side. If a new head with two eyes should appear in the new material, then the left side of this head, formed of the old material, would be in point of size out of all proportion to the new side of the head. Again only enough new material might form at first to make possible the development of one eye. If so which eye would come in? Four pieces were cut off (P. maculata), Oct. 28. Twelve days later three of these had new tissue along the cut-side.

Two of these pieces had one eye each that in one of the two was a right eye, Fig. 9 a^1. One of the three pieces, Fig. 9 *b*, had been cut just through the edge of the old eye. This eye had formed again, and a new eye had developed in the new part, Fig. 9 b^1.

Both pieces were alive a month later, one had formed a pharynx, and had changed into a small planarian, Fig. 9 b^1; the other was short and had only a single eye, Fig. 9 a^1. This piece died before Dec. 22 and the former died before Jan. 7.

Several other similar experiments were made, usually with larger pieces. When the cut was made to one side of the eye, sometimes one eye, sometimes two came in the new part. The pieces formed new worms which, after feeding, became symmetrical — the new side of the head growing at the expense of the old until a new symmetrical, but smaller, head had formed. Some of the pieces were fed, and in one experiment kept alive from Nov. 8 to March 6. Four of these worms had two eyes each, and two others had a sort of double, fused eye with a clear outer area on each side of a double pigment-mass.

The results of these experiments with lateral pieces, cut from the anterior end, show, that the new tissue at the side of the anterior end may form, in connection with the old part, a new head. The new side is at first smaller than the old part, but the former enlarges as the latter decreases until at last a symmetrical worm is formed. A piece of this sort behaves in much the same way as does a piece of the same size taken from the side at any level.

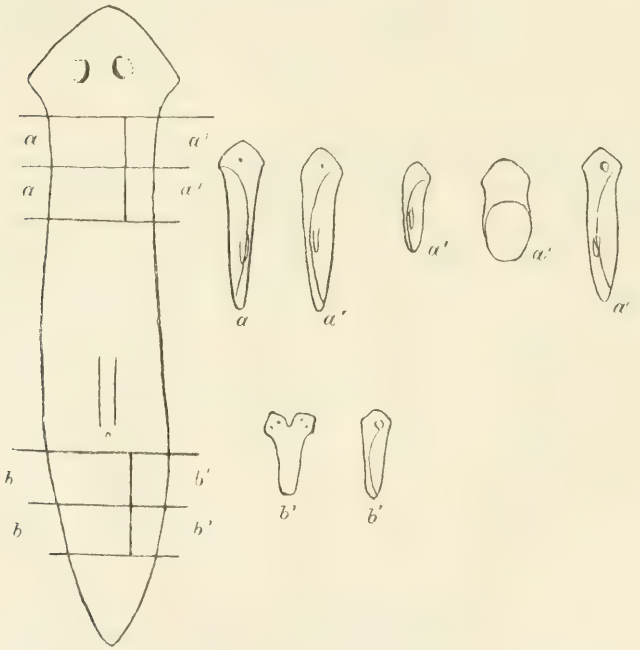
In another series of experiments I studied the development of pieces cut from the worm as shown in Fig. 10. Short cross-pieces were first removed, and each divided by a longitudinal cut into unequal parts, a and a'. The object of the experiment was to determine how much new material would form at the cut-side of each kind of piece.

In the first experiment (Sept. 16) two of the larger pieces, a, had formed a new head, after 9 days, Fig. 10 a, one of the smaller pieces, a', Fig. 10, had developed in the same way, another piece of the latter kind had a head, but no eyes as yet, and a third piece was a right-angled form. The posterior pieces, b, also produced one piece with one eye, b, and one piece with two heads, b', Fig. 10 b, b'. In another experiment of the same kind seven pieces of each sort came from the anterior end, a and a', and eight pieces from the posterior end (Oct. 1). On Oct. 13 the broader pieces, a, had very little new material at the side, and had formed a new head with two eyes at the anterior end. Of the narrower pieces, a', two had formed longitudinal worms and three right-angled forms. In the broader posterior pieces, b, very little new material had formed at

the side; one piece had only one eye. In the narrower pieces, b', the development was not far enough along to make it possible to see what was taking place.

On Oct. 23 only one piece was alive of a. It had a well developed head and a pharynx placed unsymmetrically in the posterior part. Of a', there were four right-angled forms and two longitudinal worms. The latter were long and very narrow, and the eye on the new side was smaller than the other. On b, there were three longi-

Fig. 10.



tudinal pieces with two eyes each, one right-angled form with two heads, and two pieces that had not formed heads.

On Nov. 5 there were six long pieces each with two eyes. In a' there were two longitudinal forms (in one of these one eye was smaller than the other), two without a head, and three right-angled forms. In b there were three longitudinal forms and two without heads. In b' two longitudinal and one right-angled form.

In later stages the largest longitudinal forms in a and a' became, after feeding, full-size planarians and symmetrical. The worms from a' being at each stage smaller than those from a. The same result

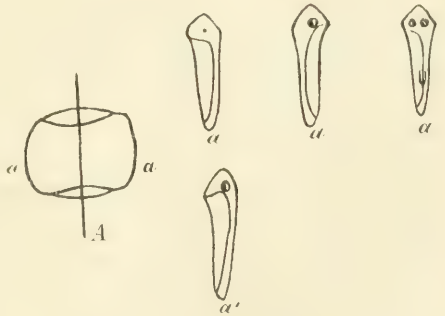
took place in the longitudinal forms in *b* and *b'*; the right-angled forms in all cases became smaller and smaller, and then died.

The main result of this experiment was to show that neither the broader nor the narrower pieces replace at first at the side anything like the amount of material removed, but the greater part of the old material is used up in a longitudinal direction. The position of the new pharynx in these pieces was not specially studied, but it appeared in the anterior pieces at one side, and near the posterior end. It lay at first unsymmetrically, but as the worm elongated it lay nearer the middle line, and, after feeding, became symmetrically placed. The number of right-angled forms is noticeably large in the narrower pieces. This shows that the head formed at the edge of such pieces from the sides is not the result of the shape of the piece, but rather due to the small amount of new material. The formation of two heads in some of these pieces may be due to the new material forming both at the anterior end of the piece, as well as at the side behind the anterior end. This will to clearer from the results of experiments described below (page 83).

Cross-pieces cut in two longitudinally after regeneration has begun.

These experiments were made in order to see, if, after the new material is laid down, it will, if divided, produce a half or a whole head or tail. Three cross-pieces were cut from each of five worms on Sept. 10. Four of these pieces were split in two longitudinally six days after the first operation. At this time, Fig. 11 *A*, new material had appeared at each end of the piece, but the eyes had not formed in the anterior part. Seven days later one eye appeared in each piece in all the pieces except one that had both eyes. Two days later five pieces had one eye each, two had two eyes, but one larger than the other, and one had two eyes, Fig. 11 *a*. Two days later the missing eye appeared in all the one-eyed pieces. It lay on the cut-side, and was smaller than the other eye.

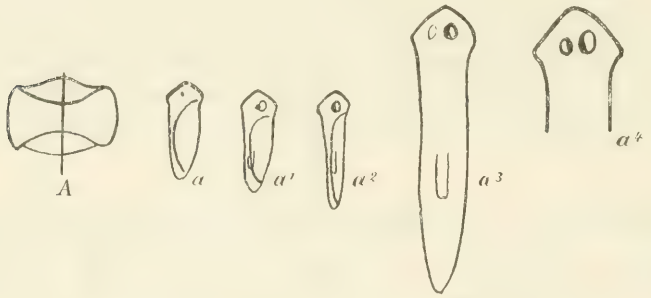
Fig. 11.



In the same experiment some of the pieces were not split in two until one day later. Six days later all the pieces (except one) had only one eye. Two days later four had each one eye, and three had two eyes. Two days later the missing eye could be seen faintly in the preceding four worms.

The experiment demonstrates that if before the eyes have appeared the piece be split in two each half continues to develop its half-structure. The whole is made good by subsequent regeneration at the side. Both parts are being formed at the same time, but the half that has a start continues to differentiate without waiting for the other. The result has many points of similarity to Roux's experiment of injuring one blastomere of the frog's egg.

Fig. 12.



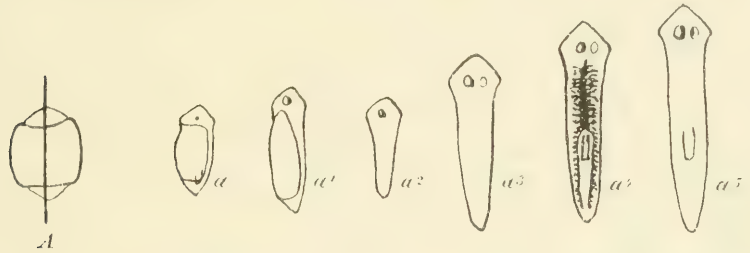
Another somewhat more complete series of experiments were made in order to see how early this bilaterality is present in the new part, or at least to determine how early the new material if cut in half still produces a single head.

On Oct. 28 two cross-pieces were cut off in front of, and two behind the region of the pharynx. On Nov. 1, when new material was visible at the anterior and posterior ends, one of the pieces (from the anterior part) was split in two longitudinally nearly in the middle, Fig. 12 *A*. On Nov. 5 each piece had formed new material at its anterior end, and a little at the side, Fig. 12 *a* (one piece). On Nov. 13 one piece had one eye, Fig. 12 *a'*; the other had not developed so far. On Nov. 26 each piece had one eye, Fig. 12 a^2; on Dec. 5 the most advanced piece had two eyes, and the other one eye; on Dec. 14 the latter had a clear area in the place where an eye should be, Fig. 12 a^3, and this condition remained until Jan. 27 when another eye had formed, Fig. 12 a^4. The results show that even at an early stage the operation delayed the development of the

eye on the cut-side; but even after the new head had been present for some time the other eye developed.

In this same experiment one of the cross-pieces from the posterior end was split longitudinally exactly into halves (on Nov. 2), Fig. 13 *A*. At the time of the second operation it was further advanced than was the preceding piece when split in two, but no eyes had appeared. On Nov. 5 one of the pieces had one eye, Fig. 13 *a*, the other was small, and had little new tissue. The former had but one eye on Nov. 9, Fig. 13 *a'*. The worm was fed on Dec. 14, Fig. 13 a^3, and at this time a clear area appeared on the head on the cut-side, but the pigment spot was not present on Jan. 7, but on Jan. 27 a minute black point, Fig. 13 a^4, could be seen, and this was larger on March 2.

Fig. 13.



These experiments give a good idea of the behavior of pieces cut in two after the regeneration has begun. The results must, of course, be checked by those in which only one eye developed in small pieces from the side. The latter experiment shows that the single eye need not, in the experiments just described, be only the outcome of halving a piece that had already begun to differentiate. While it is obvious that this possibility exists, nevertheless, it can scarcely be made to account for all the observed results; for not only were these pieces larger than the pieces described as *a'* and *b'* on page 76 Fig. 10, but the percentage of half-forms was greater. Under these circumstances, I think, the experiments must be interpreted as the result of halving the new material, and, if so, they show that very early the new material is formed into right and left halves.

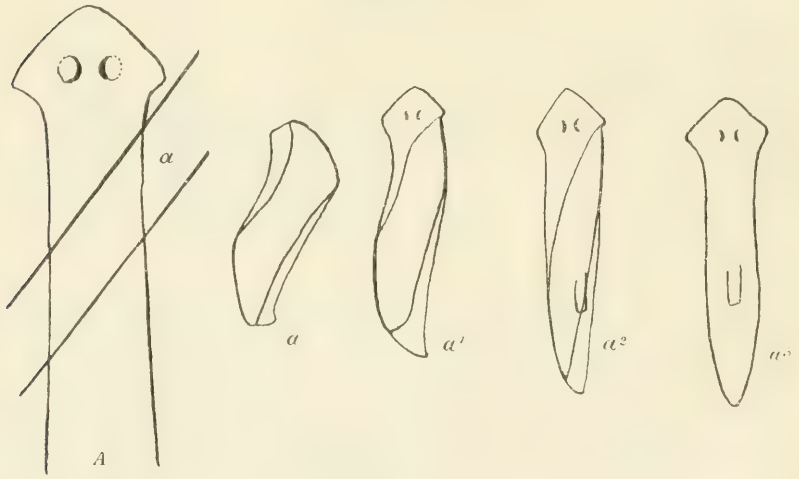
Regeneration from an oblique surface.

I shall now describe a series of experiments that have particularly interested me, and which ought, I think, ultimately to have an important bearing on the problems of regeneration. In my former

paper on »Regeneration in Bipalium« (1900) I have described similar results, and in my Woods Holl lecture of 1899 I gave some of the main points of this experiment.

If a piece be cut from a planarian by two oblique parallel cuts, as indicated in Fig. 14 *A*, the transformation of the piece into a new worm is most remarkable. A piece of this sort was cut off July 3, and by July 13 a new head had developed at the anterior end at one side, Fig. 14 *a*, *a'*. In this region the new material grew faster than along the rest of the cut-edge, and it soon became clear that the new head was forming at the side, and not in the middle of the piece. Conversely, along the posterior end the new

Fig. 14.

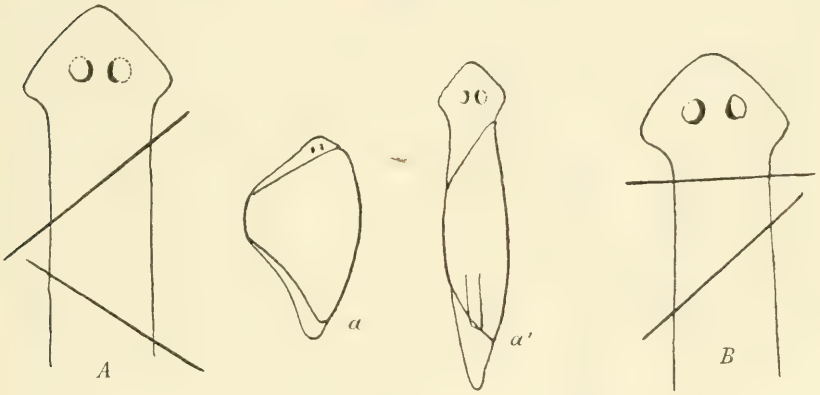


material grew faster at that side that lay nearer to the posterior end of the old worm. In this region, and again not in the middle of the piece, a new tail developed, Fig. 14 *a*, *a'*. The new head and tail appeared, therefore, not in line with each other, but on opposite sides of the piece.

The movements of the newly formed worm were at first very erratic, but as it grew straighter from day to day it moved more definitely in a forward direction. On July 17 the piece appeared as shown in Fig. 14 a^2. It will be seen that the new pharynx has appeared in the middle line of the posterior cut-edge, and, therefore, at first not in line either with the new head or tail. As the worm changes its form the head comes to lie nearer to the anterior end of the entire mass, the tail at the posterior end, and the pharynx

more nearly in the middle of the worm. It will also be seen that the greater part of the new material along the anterior and posterior cut-edges forms the sides of the body of the new worm. The piece was fed, and it filled up its digestive tract with the red pigment material of the eye of a fly. One could see then, that the shape of the digestive tract was extremely irregular, being represented by a hollow, irregular mass or sac in front of the pharynx. On Aug. 13, Fig. 14 a^3, the worm was symmetrical on the exterior. It was fed, and a new, almost symmetrical, digestive tract was seen to be present. At the sides, however, in the new parts the new tubules were still small. After feeding several times the worm grew to the full normal size and had a perfectly formed digestive tract.

Fig. 15.



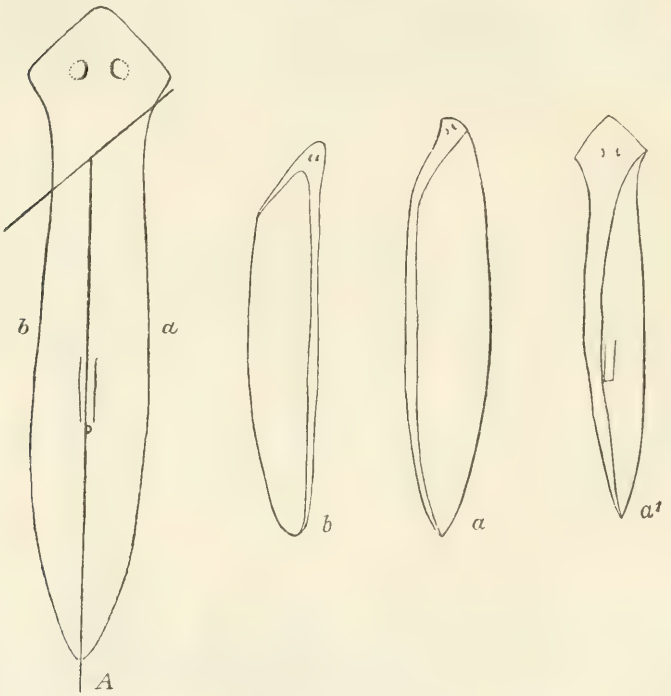
The unexpected position in which the new head and tail appeared on this piece led me to undertake a large number of similar experiments, as well as variations of the experiment with the hope of obtaining some clue as to its meaning.

The experiment just described was repeated with eleven pieces, and these all gave exactly the same result showing that the phenomenon is a constant one. The experiment has been repeated with three other species, P. sp. ?, Phagocata and Bipalium and the same result obtained.

If, as the experiments seemed to show, the shape of the piece is the factor that determines the position of the new head and tail, then if a piece is cut out by two oblique cuts making an angle with each other as shown in Fig. 15 *A*, we should expect to find, that the new head and tail would again appear at the side, but, in this

experiment, both on the same side of the worm. Numerous experiments of this sort have shown, that this actually happens as shown in Fig. 15 *a*, *a'*. Finally if we cut out a piece by one cross-cut and one oblique cut, as shown in Fig. 15 *B*, we should expect the new head to appear in the middle line of the anterior cut-edge, and the new tail at the side of the posterior cut-edge. An experiment of this sort gave the expected result. Conversely by making the anterior

Fig. 16.



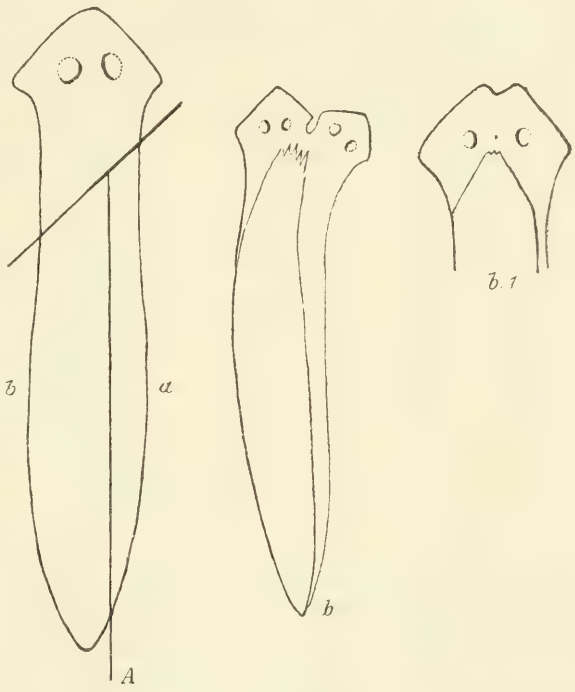
edge oblique, and the posterior edge a cross-cut the relations of the new part changed accordingly.

By means of these experiments we can change, at will, the position of the new head and the new tail from the middle to either side by making the cut oblique in either direction.

The following results give another variation of the experiment. As shown in Fig. 16 *A* the worm was first cut in two by a cross-oblique cut; then the posterior end was split longitudinally into two equal parts, *a* and *b*. We should expect to find the new head in *a* appear on the right side of the cut-edge. This happened, as shown

in Fig. 16 *a*. For *b* there are two possibilities. The new head might appear on the inner side of the anterior oblique edge, since this is its more anterior part, or it might appear on the anterior end of the middle cut-edge. In the first experiment the new head appeared on the anterior cut edge at its inner side. The same experiment repeated on another worm gave, in the piece *a*, a new head at the outer edge as before, although it occupied most of the cut surface. In the piece *b* the new head appeared opposite the anterior angle, Fig. 16 *b*, and arose most probably from material derived from both the anterior oblique-edge and the anterior end of the middle cut-edge. In a third experiment the new head in *a* appeared distinctly at the outer edge; in *b* a single head appeared as in the last experiment.

Fig. 17.

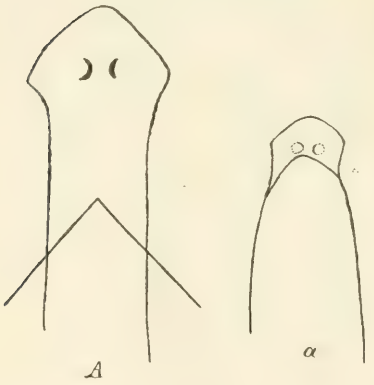


Another variation of the experiment consisted in cutting the worm in two by an oblique cut as before, and then splitting the long posterior piece into two unequal parts, Fig. 17 *A*. In the single experiment of this kind, that I carried out, on four worms, the right piece *a* was the smaller. This piece produced a head at the anterior end, but the piece was so narrow that the new head occupied almost the entire width of the anterior oblique surface. The larger piece, *b*, produced in two cases a single head, but I failed to note from which part of the cut-edge it developed. The third piece produced two heads, Fig. 17 *b*, one on the inner part of the anterior oblique-edge, and the other at the anterior end of the inner edge.

The fourth piece produced a single head with indications of a double structure. The anterior end had two points, Fig. 17 b^1, that did not disappear, and a single median eye-speck was present between the two large eyes. The third experiment of this series shows us that in reality the possibility of two heads forming in the piece b may be carried out.

These experiments taken all together bear out in a most striking way the deductions drawn from the first experiment. The head appears always at the most anterior edge, regardless of whether the cut-edge is at the side (Fig. 16 a), or in the middle of the worm (Fig. 16 b). The results show further in a case like that in Fig. 16 A, b, in which several possibilities exist, that all these may be realized. In most cases of this sort the material from both the anterior, and the median cut-edge unites to produce a single structure. This shows how far the phenomena is an epigenetic one. Either one or two heads may develop depending, in all probability, on some slight, perhaps trivial, initial difference in the form of the anterior cut-edge, or the rate, and kind of accumulation of new material near that point.

Fig. 18

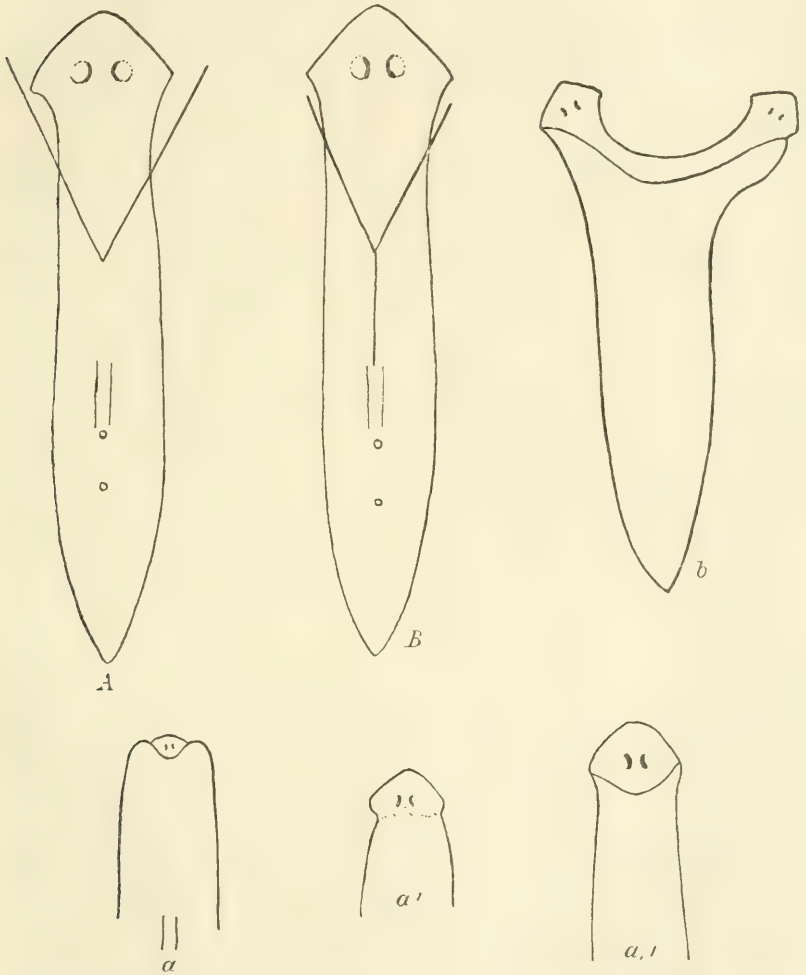


Two further variations of this experiment readily suggest themselves. If the worm is cut in two by two oblique cuts that meet in the middle line, Fig. 18 A, we should expect to find one or two new heads appearing at the apex of the posterior piece. In several experiments of this sort a single head appeared at the anterior middle point, Fig. 18 a.

The other variation consisted in cutting the worm in two by two oblique cuts as shown in Fig. 19 A. Several possibilities are present for the posterior piece: a) a new head might appear on the inner cut-edges of each side; b) a single head might develop between the two sides; c) the edges might come together and fuse in the middle line. In this experiment fourteen worms were used, each developed a single head in the middle line between the two side-pieces, Fig. 19 a, a', and a^1. It may seem that this result is contradictory to the results of the preceding experiments, but there

are some additional facts to be considered. The pointed ends contract very much after the operation, as shown in Fig. 19 *a*, so that the new material that forms at the cut-edges is continuous across the middle line. Out of this the new head develops. The material

Fig. 19.

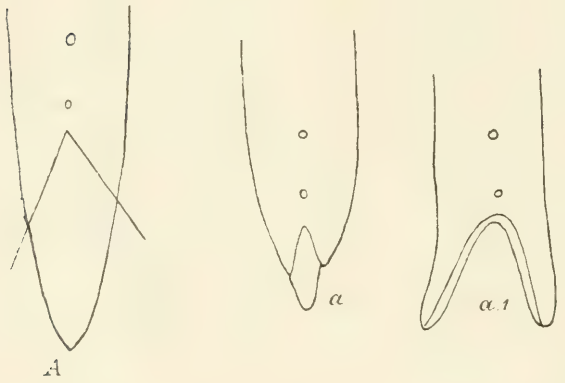


of the new head arises, therefore, out of material derived from the two sides, but the fact that the material of the one side is from the beginning connected with that from the other side accounts for the appearance of a single head. If the two edges could be kept apart then two heads ought to develop. It is possible to accomplish this

by splitting the worm in two for a short distance after the operation as indicated in Fig. 19 *B*. Five worms were operated upon in this way. In all of these, but one, the edges came together again and only one head formed. In one worm the edges met in part, but the two sides were kept far enough apart for two heads to appear, one on the inner cut-edge of each half, Fig. 19 *b*. If the cut had been kept open in all the worms by repeated separation, there can be little doubt that a larger number would have formed two heads.

In order to see if two tails could be produced by a similar experiment a worm was cut in two as indicated in Fig. 20 *A*. In four worms the large anterior pieces formed only a single median tail, Fig. 20 *a*, but in the fifth piece the two sides kept further apart and each produced a tail, Fig. 20 *a*[1]. Randolph has obtained similar results.

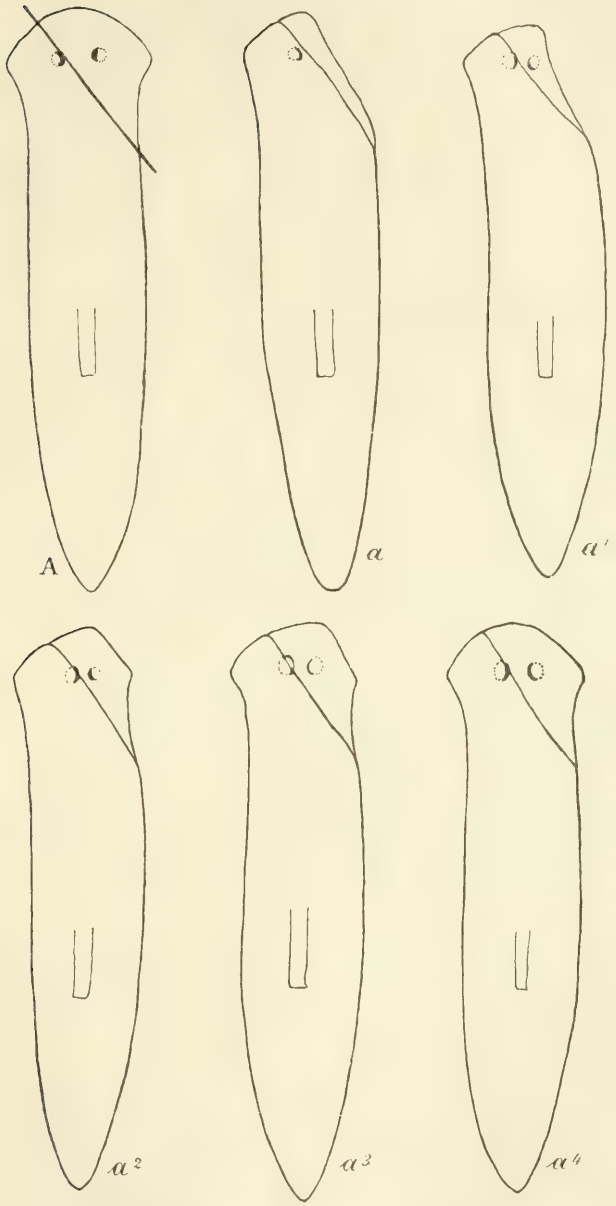
Fig. 20.



It seemed to me that some light might be thrown on the appearance of a new head at the side of oblique cuts by the following experiment.

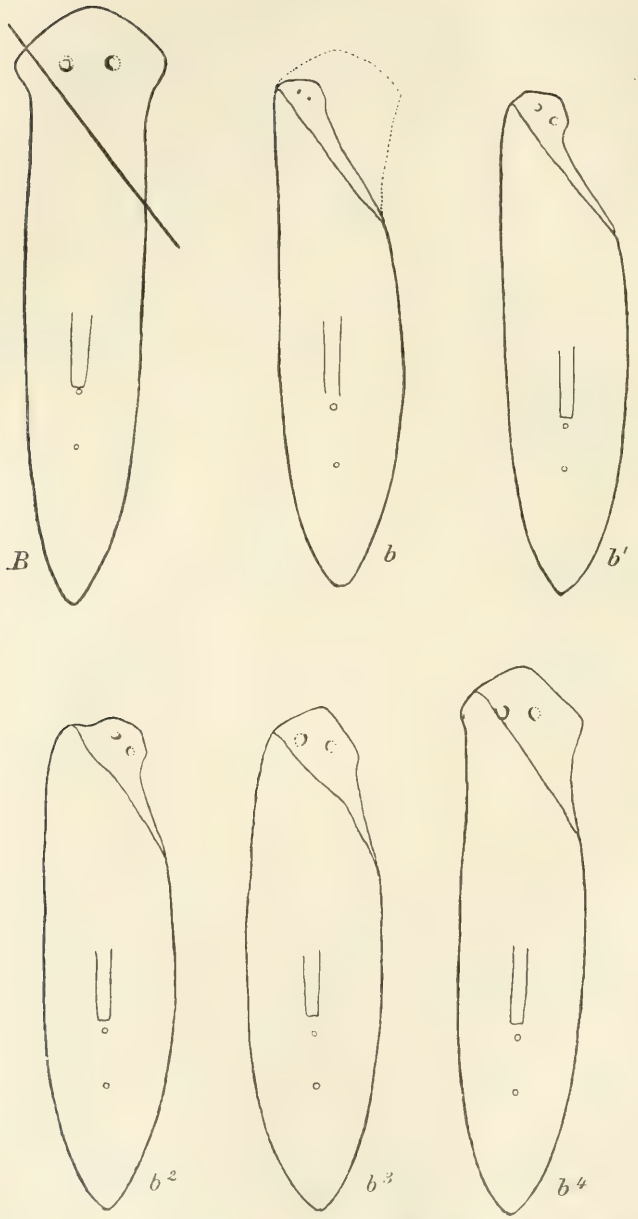
The worms P. sp. ? were cut in two by an oblique cut through the head as shown in Fig. 21 *A*. The cut passes through one eye. If in this case a new head were to develop at the highest cut-edge a worm would be produced having an entire, new head, and a part of the old one just behind it. I did not, in fact, expect this to happen, but rather that the missing half of the new head would appear. As shown in Fig. 21 *a*—*a*[4], the new material at the cut-edge formed along its more anterior part the missing portions of the head, and along the rest of the cut-edge the side of the body in that region. This is even more evident in the later stages, Fig. 21 *a*[2]—*a*[3]. It will be noticed that the new head stands at first somewhat obliquely to the long axis of the worm, i. e., more or less at right angles to the cut-edge.

Fig. 21 *A*—a^4.



Another experiment somewhat similar to the last supplements it, and shows more clearly the oblique position of the new head. In this experiment the head was cut off by an oblique cut as shown

Fig. 21 *B*—b^4.



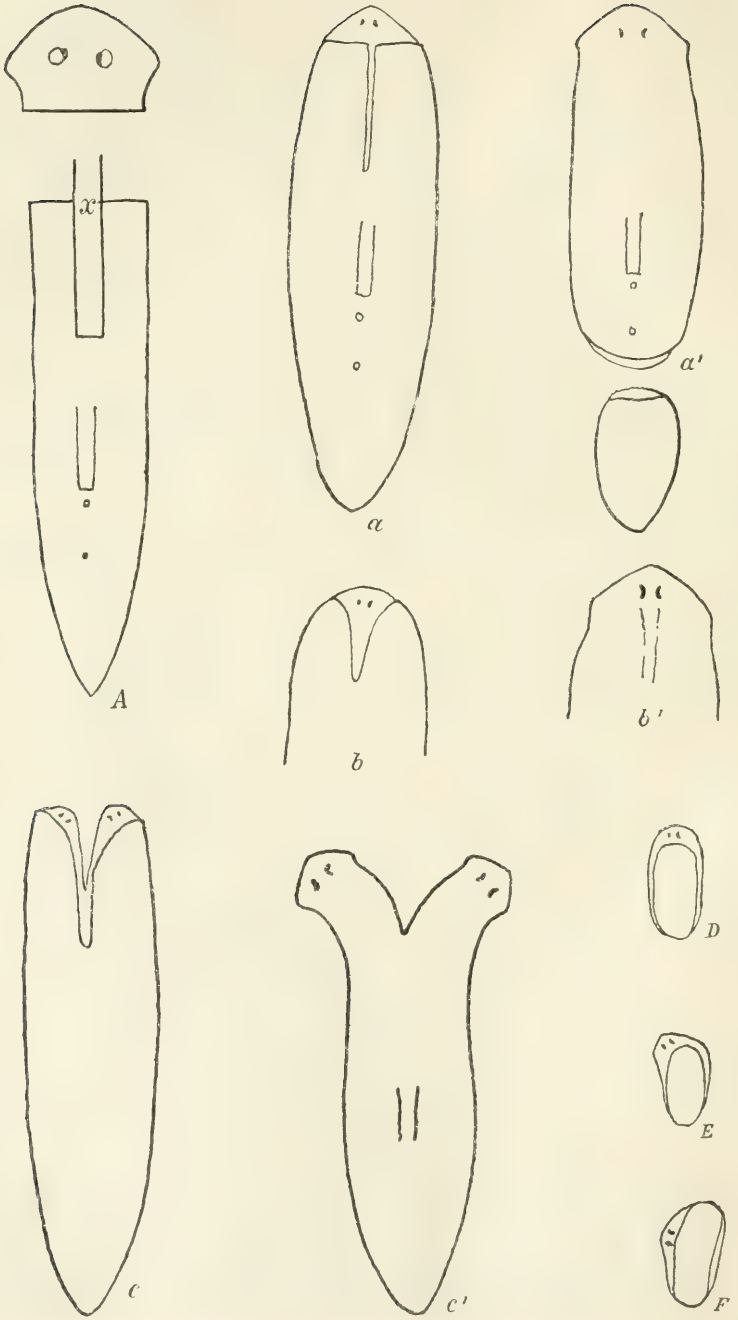
in Fig. 21 *B*, the cut passing on the left side just above the ear-lap, but on the right far down that side of the body. The presence of the ear at one side gives a fixed point near which we should

expect to find the new head. This will be one factor determining the position of one side of the new head. The new material appears along the entire cut-edge. Even if the new head were as broad as the old one (in fact it is always less) it would use up only a part of the entire length of the oblique surface, which is of course greater than the breadth of the worm. One point of the new head being fixed at the left side, the head must, therefore, lie along the left side of the oblique edge, and since it is less in breadth than the entire length of the cut-edge its median plane will lie at one side of the median plane of the body. The rest of the new part will be left over to form the side of the body. I shall discuss this question more fully in the general part of this paper.

In the above analysis it is assumed that any part of the new material formed at the cut-edge may potentially produce a head, and that something in the old part determines where the new part is to form. That the new material has this potentiality is demonstrated by the following experiment.

The head was first cut squarely off and then two longitudinal cuts were made along each side of the middle line, and the middle piece, *x*, cut off, Fig. 22 *A*. In most cases the sides came together in the middle line. The anterior cut-edge is therefore narrower than the former width of the worm. A seam or scar marks for some time the median line along which the fusion of the sides took place. The new material that arises at the anterior cut-edge will come necessarily only from the old material that lay at the right and left sides of the median plane; the old material of the middle part having been removed with the median piece, and the formation of a scar along the line of fusion will prevent or delay the forward migration of median material from behind. I awaited with some interest the outcome of the experiment, for, if the source of the new material had any direct influence on the new part, as did not seem probable from other experiments, the median part of the new head would be absent. The new material formed in the usual time, and a single head appeared complete in every respect, Fig. 22 *a*, *b*. It developed in the same way as when the median part is present. This experiment is, I think, of some theoretical interest since it shows, not only that the head may develop from material derived solely from the sides, but that the presence of the old material in the middle is not an essential stimulus to make the middle part of the new head. The same result had been obtained in a somewhat different way in the obliquely-cut pieces.

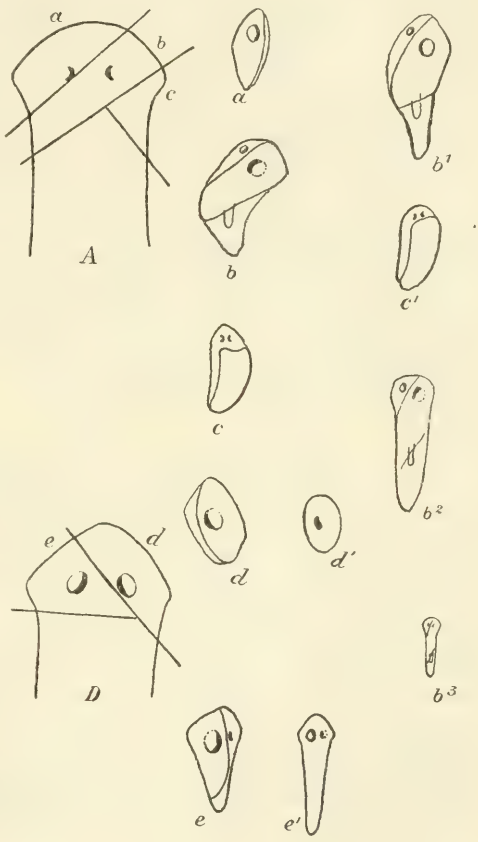
Fig. 22



As the head grows larger a lighter area, of apparently new tissue, appears along the middle line previously occupied by the scar. The lost middle region slowly comes back, but not for some time after the new head has formed.

In several other worms in this experiment the sides did not come together in the middle line so fully as in the cases just described, but remained for a part of their length separated from each other. New material appeared at the anterior edge of each half, and also along the inner edge. From the former a head developed in each half, Fig. 22 *c*, *c'*. In one of these two headed forms one side was smaller than the other and on this a smaller head was present. It never grew to the size of the larger head.

Fig. 23.



These two headed forms show that a symmetrical head may develop out of new material derived entirely from the side of the worm. If, however, the two sides first fuse together then the same material that formed two heads now forms but a single head. The result fully demonstrates the potentialities of the new material, and our special problem resolves itself into determining what the factors are that come into play in each case. The pieces that were cut out of the center of these worms produced new worms, Fig. 22 *D*, *E*, *F*. The piece became shorter and more rounder. New material appeared over the entire edge. In one piece a new head appeared at the anterior end of the piece, Fig. 22 *D*; in another the new head was somewhat on one side,

Fig. 22 *E*; and in a third piece the new head appeared well around the anterior end on the side of the piece, Fig. 22 *F*.

A few further experiments with oblique pieces from the head may be here briefly mentioned. The head of P. sp. ? was cut into three pieces by oblique cuts as shown in Fig. 23 *A*, *a*, *b*, *c*. The piece *a* made a little new tissue at the side, but did not develop further; the piece *b* made a new eye on the upper side and a new tail at the posterior left side. The tail stood at first sharply at right-angles to the cut-surface. From this piece a small worm, b^2, was formed, that later decreased in size, b^3, through lack of food. The piece *c* made a new worm as shown in the figure; the new head coming in somewhat on the side of the piece.

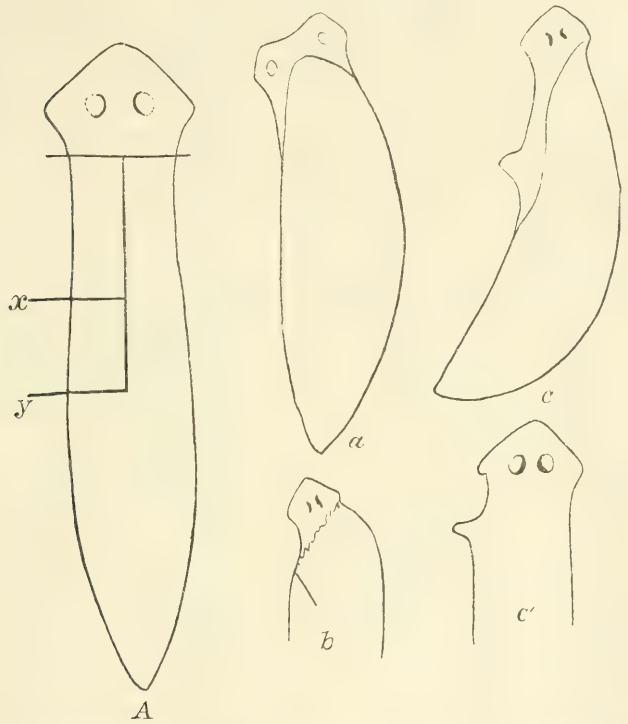
A similar experiment was made with P. maculata as shown in Fig. 23 *D*. The piece *d* was like the piece *a* in the last experiment, and behaved in the same way. The triangular piece *e* changed its shape very much and made a new worm as shown in Fig. 23 *e*, *e'*. The results show that material from the old head may also be worked over to form a new worm. Cross-sections of the head region give practically the same result.

Cross-cuts made at two levels (Fig. 24—25).

In some of the preceding experiment in which two heads were formed at the anterior cut-edge of each side (Figs. 22 and 19), both edges lay at the same level. In the following experiment, I tried to discover whether, when the piece is cut off so that one exposed edge is anterior to the other, one or two heads will develop. The anterior end of a worm was first cut off, then the posterior piece was split down the middle, and one side cut off by a cross-cut, as indicated in Fig. 24 *A*, at *x*. The half-piece left attached shortened, and at its pointed inner end a head developed, as shown in Fig. 24 *b*. The experiment was repeated leaving the attached piece less than half the width of the worm, but the piece shortened, bent inwards, and only a single head developed as before. In another experiment the worm was first cut in two nearer to the head; it was then split down the middle line for a longer distance and one half removed, Fig. 24 *A*, at *y*. Five of these pieces produced a single head at the anterior end of the half-piece, Fig. 24 *b*. The half-piece having bent over closed the exposed anterior edge at the more posterior level. Three pieces produced a head at the anterior end of the

cross-piece, and an outgrowth near the posterior exposed end, Fig. 24 *c*, *c'*. This outgrowth did not produce later any recognizable structure, and I could not decide whether it was an undeveloped head or a tail. One of these worms produced a two headed form, Fig. 24 *a*. A single eye appeared at first in each head, and later it seems that two median eyes appeared, although my notes are obscure on this point.

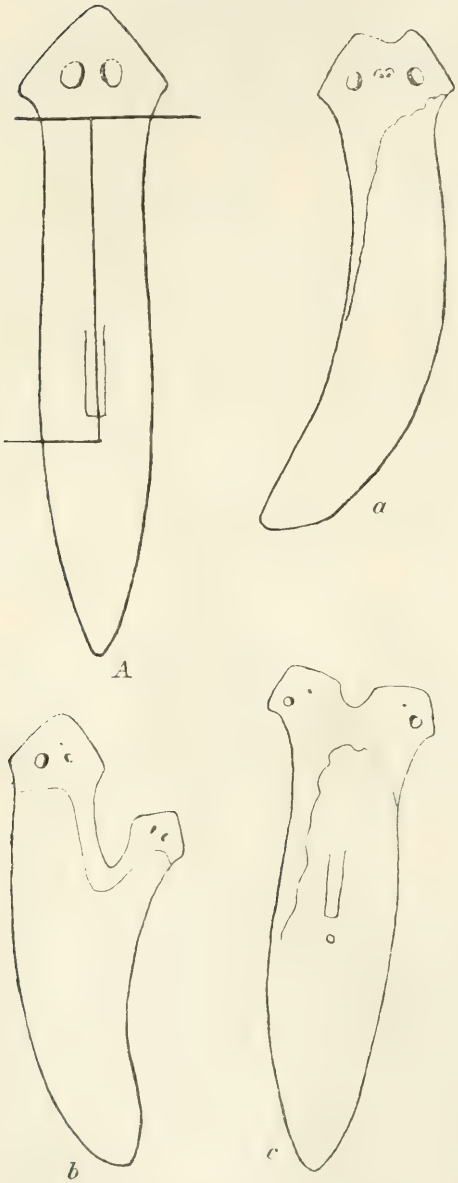
Fig. 24.



In another experiment the worm was split posteriorly behind the pharynx, and then as before one half removed, Fig. 25 *A*. In this way the anterior and posterior exposed ends were far apart. Two pieces produced a single head only at the anterior end, one produced two partially fused heads with an eye in each and a double median eye, Fig. 25 *a*; another showed some evidence of the head being double; and another produced a head at the anterior edge and a second head at the more posterior edge, Fig. 25 *b* (the right side having been removed). The anterior head was larger and on one side there were two eyes. The posterior head was smaller and the outer eye larger than the inner one.

In another experiment, like the last, two heads were formed in one worm out of five at the anterior end, Fig. 25 *c*. In this last experiment the double heads appeared at the same level, so that we can not refer one to each of the exposed anterior half-ends. The doubling is due rather to the presence of an anterior exposed half-edge and a lateral median edge. It may be that some of the material that forms at the latter edge has grown forward from the posterior exposed edge. In the worm represented in Fig. 25 *b*, the posterior head seemed to come from the posterior exposed edge.

Fig. 25.

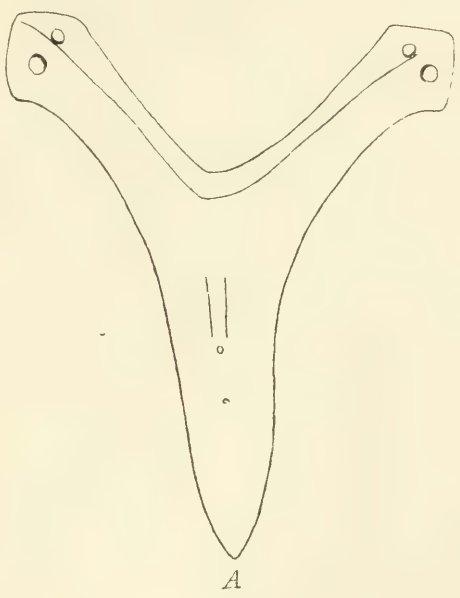


I have already described (1900) experiments, similar to these, made on Bipalium; and in this species I obtained only one head. The point of the experiment is to determine if the development of a head at a more anterior level will inhibit the formation of a head at a more posterior level. The case described above, Fig. 25 *b*, shows that this is not the case, but the experiment is in general unsatisfactory owing to the shortening of the anterior half-piece in the first experiments, and to a fusion of the posterior exposed edge in the several other experiments.

Partial longitudinal splitting.

The production of double headed forms, by splitting the worm longitudinally, has been known for a long time. RANDOLPH ('97) in a critical review of the literature has pointed out that DUGÈS in 1828, and again FARADAY in 1832 produced two headed forms in this way. VAN DUYNE in 1895 and RANDOLPH [1]) in 1897 obtained the same result. In connection with the preceding work I have repeated this experiment. If a worm be partially split in two beginning at the anterior end the halves come together again in most cases, and fuse, especially if the cut is a short one, but sometimes the halves spread apart, or overlap, and each develops a new half as shown in Fig. 26 *A*. Sometimes the halves only partially fuse, and the free ends form two heads, or, if the fusion extends further forward, a single abnormal head with additional eyes may develop.

Fig. 26 *A*.



A

If the worm be partially split longitudinally, beginning at the posterior end, the halves often remain separate, and produce new tissue along the cut-edge as RANDOLPH has shown. If the cut extends forward in front of the pharynx a new pharynx appears in each half between the old and new tissue. The new material increases in amount until the pharynx may come to lie in the middle of each posterior half-part.

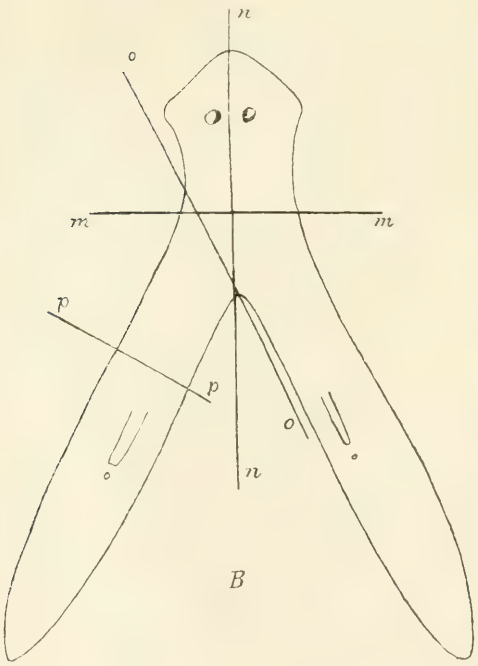
Several worms that had double posterior ends or tails were operated upon as follows. One was cut across, as indicated by the line *m*—*m* in Fig. 26, *B*, anterior to the region of forking. The experiment was made in order to see if the presence of a double

[1]) RANDOLPH's experiments were made in 1893, but not published until 1897.

structure behind the level of the cut would have any influence on the formation of the head at the anterior cut-edge. A single head, and not two, appeared.

Another of these double worms was split in two longitudinally as shown in Fig. 26 *B*, *n*—*n*. Each half produced a new half-head and half-side and each piece formed a normal worm. In a third experiment one posterior half was cut off as shown in Fig. 26 *B*, *o*—*o*. One piece contained the old head. It did not produce a new tail in place of the one cut off, but completed itself, so as to form a normal worm. The smaller piece produced a new head at the cut end. Another double worm was cut in two as shown in Fig. 26 *B*, *p*—*p*. The larger piece regenerated posteriorly and made a short tail-like structure in place of the one removed, but it had not made a new pharynx at the end of twelve days when the experiment came to an end[1]). The small piece made a new head.

Fig. 26 *B*.

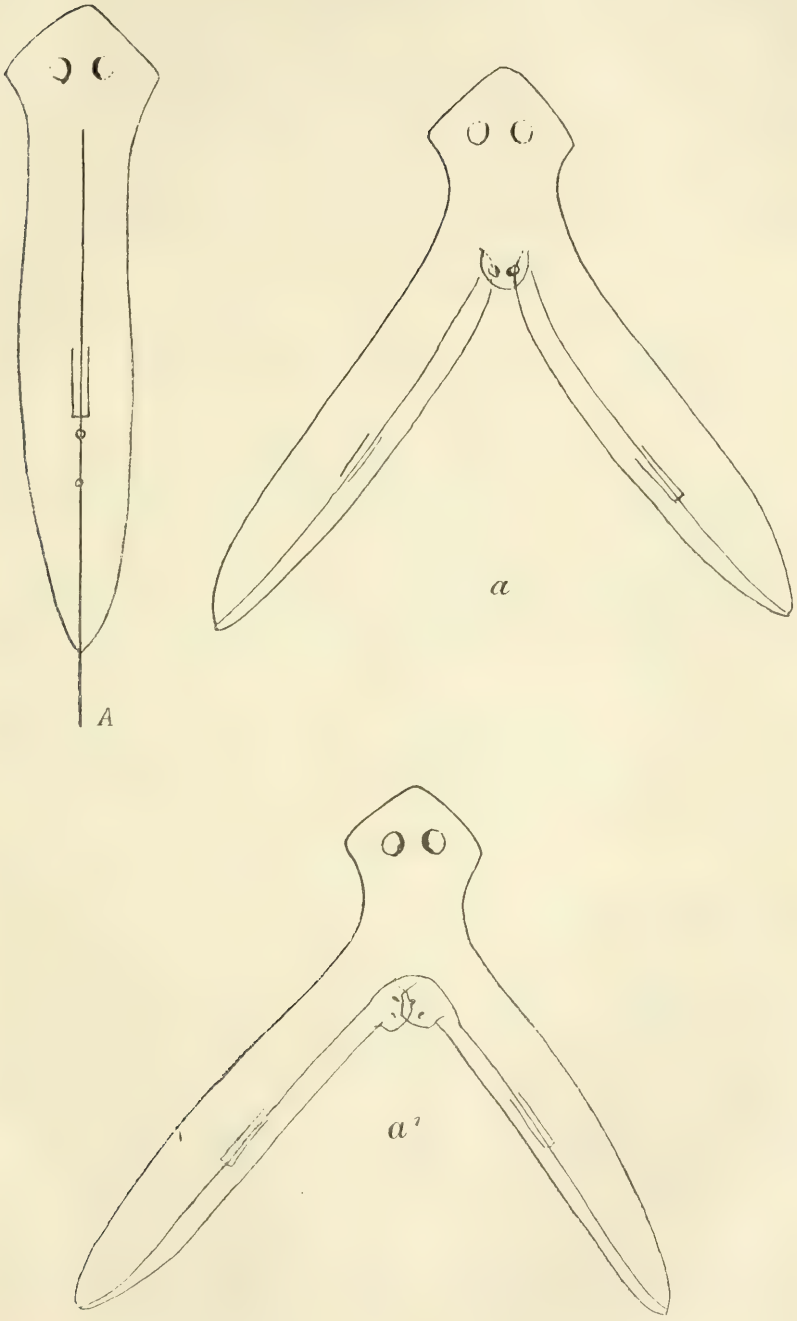


If the worms are split further forward, Fig. 27 *A*, than in the last experiments a different result is obtained. There is formed at or near the fork on the inner side one or two new heads, Fig. 27 *a*, *a*[1]. If two heads form they lie at the anterior end of the new material of each side, and point towards each other. If only one head is formed it may lie in the fork. It may happen that in some cases the single head belongs only to one side, there being no head on the opposite side.

VAN DUYNE has figured worms of this sort having one or two

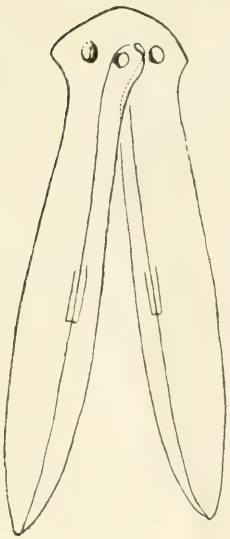
[1]) The experiment should be repeated.

Fig. 27.



heads. He spoke of these heads as arising by heteromorphosis. In my former paper I tried to show that another interpretation may be given to this phenomenon, viz., that the new heads develop in connection with the new material of the half, and appear at the side, as does the new head in longitudinal pieces, and are, therefore, not, in one sense, heteromorphic productions. It is, I believe, permissible to interpret the single median head in the same way, viz., as the result of the union across the middle line of the new material of the two sides. The result would be parallel to that represented in Fig. 19.

Fig. 28.



In another planarian (P. sp. ?) that is more sluggish than the former (P. maculata). I have been able to carry the same experiment one step further. If the longitudinal cut be extended forward between the eyes the worms do not, in some cases, pull apart. Instead of forming, under these circumstances, one or two new heads at the sides each half head completes itself more or less perfectly as shown in Fig. 28. Two almost complete worms are formed, united by a narrow strip of tissue at the anterior end. These pieces crawl with great difficulty, and it often looks as though the pieces would pull apart, but as soon as the tension on the connecting band becomes too strong, the rest of the piece, by a sort of adaptive reponse, ceases pulling in its former direction. The special point, that I wish to call attention to in this result, is that although united the halves regenerate as though they were completely separated; the factors regulating the regeneration are not altered by the union of the pieces in front of the regenerating regions. If it is justifiable to apply the same interpretation to those cases in which two new heads are formed in the fork (Fig. 27), the heads can be accounted for in the way that I have intimated.

One further point should be stated. The two heads (Fig. 27) appear, so far as my experience has gone only when the worm is split far forward in the head region, or rather in front of the pharynx. It may seem that the nearness to the old head may be the critical factor determining the production of the new head. While I am in-

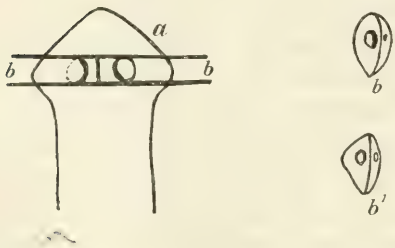
clined to think that the new head might be more readily produced from tissue in the head region, yet the fact remains, that regions much posterior to this level do produce heads if the pieces are separated, and that the cut need not pass into the old head but may end some distance behind that structure. That the new heads are only produced when the worm is split some distance forward, I attribute as due to the presence of the old pharyngeal region (to state the point somewhat crudely) in each piece. In this region the new pharynx begins to form, and the presence of this structure would, I think, prevent the formation of a new head behind that level. Theoretically it would be necessary for only a short region in front of the pharynx to be present in order to produce a new head at the side, and while I can not speak definitely on this point, yet the experiments have seemed to show that the new heads do not come in unless the piece be split much further forward than this. In other words some other factor is present that I can not account for, yet I do not think that it can be a phenomenon of heteromorphosis, at least the factors that induce the formation of a new head in this experiment are, in my opinion, different from those that produce a head on the posterior end of a piece (Fig. 30). I hope to demonstrate that heteromorphosis in the latter sense does occur in some planarians, and to show that even a head may develop on the posterior end of a piece, but I do not think that these results belong to the same category as those described for the split-worms. I can only state that in P. maculata I have not been able to produce heteromorphosis, sensu strictu, in the region in which these heads are readily formed after the operation of splitting.

Minimum Size.

In my former paper I discussed the problem of the minimum size of pieces capable of regenerating. The results showed that below a certain size a piece does not form a new worm, although it may remain alive for some time, and be derived from a part of the body capable of regenerating. I found that there is some difference, as was to be expected, in different regions of the body. For instance the region in front of the eyes, if cut off, does not make a new worm, although a piece of the same size taken from the side or from the tip of the tail regenerates. Several times during the past summer I have repeated this experiment, and have obtained

the same result[1]). A variation of this experiment consisted in cutting off a very short cross-piece through the region of the eyes — shorter than the region in front of the eyes — and then splitting the piece in two between the eyes, Fig. 29. In this way a smaller piece than that anterior to the eyes was obtained. The pieces lived longer as a rule than the anterior pieces, Fig. 29 *a*, although they had exposed edges along three sides. A few pieces developed new tissue along the cut-side, and a new eye appeared. These pieces grew longer antero-posteriorly, although I did not observe the formation of a new pharynx. The experiment shows that in the region of the eyes a piece no larger than that in front of the eyes may form a new worm, although in the cases observed the small worms died before the process was entirely completed.

Fig. 29.



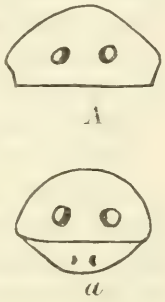
There is an interesting theoretical question connected with this limitation in the power of regeneration in small pieces. Elsewhere ('99) I have pointed out that we find the same limitation in the power of development of isolated blastomeres, and, as Lillie has shown, it exists also in the Protozoa. Naturally one asks, has this any thing to do with what we may call the organisation unit of each organism? Do experiments of this kind show us the size of the unit of organisation of the material of which each animal is composed? If this could be established it would give us, I think, a result of the greatest interest. The experiments on these planarians show also that while a smaller head may form on a smaller piece than on a larger one, yet there is a lower limit beyond which a new head does not form. It is the same problem that Peebles ('97) has studied in Hydra where a piece below a certain size was shown to be incapable of remoulding itself into the typical form. If this is the correct way of looking at the result it is a curious fact that any piece larger than this unit forms only one single individual. At another time I shall hope to discuss this question more adequately.

[1]) I wished particularly to do this since in my former paper some of the figures (Figs. 13, 14, 15, 16) were by an error drawn to too small a scale in comparison with the other figures (Figs. 11, 12). The measurements given in the text are, however, correct, as well as the conclusions.

Heteromorphosis.

In my former paper I described one case in which a narrow cross-piece of P. maculata developed a head on each of the opposite ends. Numerous subsequent attemps to discover the special conditions that produced this result led to no definite results. With another planarian, P. sp. ?, I have been more successful. If the head of this planarian is cut off just behind the eyes, Fig. 30 *A*, a large percentage of the pieces produce another head, and not a tail, on the posterior end, Fig. 30 *a*. If the head is cut off more posteriorly, then all the head-pieces form a tail. The results show that the phenomenon is in some way connected with the cut-edge passing through the old head. I have made serial sections through one of these two-headed forms, which show that the new part is a perfectly formed head with brain, nerve cord, eyes, etc.

Fig. 30.



This experiment makes it probable, I think, that the double headed piece of P. maculata that I described ('98) came from near the anterior end of the body near the old head, but even under these circumstances the result must be unusual, as I have not obtained a two headed piece in several experiments in which I removed short cross-piece from near the anterior end.

The more theoretical questions connected with this case of heteromorphosis will be discussed in the following general part.

Theoretical.

Morpholaxis.

In many of the experiments described in the preceding pages attention has been drawn to the remarkable changes in shape of the old part during the development of a new worm. The change is most easily followed in a cross-piece, Fig. 3. The new material that appears in the course of two or three days seems to arise not so much by multiplication of the cells exposed at the cut-surface, as by an active migration of old tissue. As a necessary result the old part decreases in breadth, and in height. The new material differentiates at once into a new head and tail. At this time the worm

is much too broad for its length. The migration of tissue forwards and backwards, especially forwards, does not stop with the production of a new head and tail, but continues, and in consequence the head is carried further forward and the tip of the tail further backwards. This process goes on until the proportion of length to breadth is approximately that of the typical form. If now the worm be fed it grows in all its parts, and may, in the course of a short time, reach the adult size. If it is not fed it slowly decreases in size until it finally dies.

If the planarian is cut in two in front of the genital pore the pushing forward of the new head on the posterior piece can be more readily followed, since the genital pore gives a definite landmark. The new head forms, out of the first new material that appears, immediately in front of the pore. New material continues to push forward in the region in front of the pore and behind the new head, so that the latter is continually being carried further forward. It is noticeable that the first new material that appears is whitish and without pigment (and the new head develops out of this lighter colored material), but the material that continues to appear behind the new head is darker, and one gets the impression that the old material is extending forward. The presence of a fixed point, the genital pore, shows, however, that the change just behind the head is not so much a remoulding of the old part as an active migration forward around the pore of the old material.

The lateral regeneration is equally interesting. It has been shown that when the worm is cut in two longitudinally, Fig. 5, new material appears along the entire cut-edge. The new pharynx appears at the level of the old pharynx at the edge of the old part, or just within the old part. It is for a long time unsymmetrical in position. The new material at the side accumulates rapidly until it is about one-third the breadth of the old part. The old part has been decreasing in breadth as the new has increased. The new part forms at the expense of the old and appears to develop, although I have no definite proof of this, out of the tissue of the old part that migrates laterally. A puzzling fact in regard to these pieces at this stage is that as they crawl forward they appear perfectly symmetrical in outline. The head appears to stand in the middle of the anterior end of a bilaterally symmetrical body. It is only when we examine the structure of the digestive tract, and the position of the pharynx, that we notice that the worm is still quite unsymmetrical. The

external symmetry may be deceptive, but the outline of the worm certainly appears bilateral in form at a stage when the internal assymmetry is most marked. If the worms are not fed they decrease in size, and the rectification of the internal assymmetry goes on very slowly. If the worms are fed they increase in size, and the new side grows faster than the old, so that the body soon becomes quite symmetrical. Branches from the old parts of the digestive tract extend out into the new side, and the digestive tract becomes bilaterally symmetrical.

The position of the new pharynx that first appears at the border of the old and the new parts is a fixed structure in the new worm, and around it the subsequent changes take place. This only means that the pharynx is, for us, the most obvious central point, for throughout the entire length there is a similar median line or structure; in fact the central part of the stomach extending forward in front of the pharynx can be seen to occupy the line along the old and new parts.

In a long piece cut to one side of the median line the new pharynx comes in also along the border between the old and the new parts, and the new tissue at the side continues to increase at the expense of the old until a symmetrical worm is formed. I have noticed that for some time after these narrow pieces have become symmetrical, or nearly so, some of the new worms are much too long for their width. If fed they grow broader and after a time a normal worm may result. It would seem that such pieces most grow in the later stages much faster in breadth than in length. If the worm is not fed I do not known whether they will decrease in length faster than in breadth (or continue to grow broader at the expense of their length) and attain more nearly the normal proportions. The problem needs to be more fully studied.

On the basis of these results in regard to lateral regeneration it is possible to add certain points to the facts discovered by Randolph in this connection. Randolph noticed that if a worm be split longitudinally into unequal parts that »if less than one half of the body be removed by either a transverse or a longitudinal cut there is a restoration of the amount that has been lost«. The statement leaves out of account that the new tissue forms at the expense of the old, and hence in order to establish the symmetry, or the normal proportions it is only necessary to form somewhat less than the part removed, since the old part is decreasing in size at the same

time. The same modification may be made of the statement that in »pieces one-third and one-fourth the width of the whole animal; the new individuals are respectively two-thirds and one-half the width of the original worm«. The measurements that I have made of such pieces show that in actual breadth the pieces increase very little, if at all, since the old part loses what the new part gains.

If we examine the regeneration of pieces taken from the side that are less than the entire length of the animal, we find that the amount of new material at the side is smaller than in the preceding cases, as RANDOLPH has shown. The old part gives rise to a relatively large amount of new tissue at the anterior and posterior ends, and in consequence the loss in breadth may be very great. Even when the pharynx appears at the side of such a piece between the old and the new parts the antero-posterior extension of the old material will so far reduce its amount that a relatively small amount of new material on the new side will establish the symmetry.

The changes that take place in the old part of pieces cut obliquely from the body, Fig. 14, are more difficult to follow. As has been stated the new head forms at one side of the anterior oblique edge, and the tail forms also at the side of the posterior edge. The new pharynx comes in, in all cases observed, in the old middle line near the posterior edge. The changes that follow are extremely difficult to understand. I have attempted to make them out, as far as possible, in the living animal. As the figures show the head continues to get larger, and is pushed somewhat further forward, Fig. 14. The old tissue in the middle line at the anterior cut-edge seems to extend forward, and bend over towards the middle line of the new head. At the same time the old piece is changing its form, so that it comes to lie more in line with the new head and tail. It is noticeable that only a small amount of new tissue forms along the part of the oblique edge that is at the side of the head, and only a small amount along the posterior edge outside of the new tail. This new tissue forms the greater part of the side of the body of the new worm. It is difficult to find out how much of the change in shape of the old part is due to migration of material forwards and backwards, and how much to remoulding of the old material in situ. My observations indicate that extensive changes of both kinds take place.

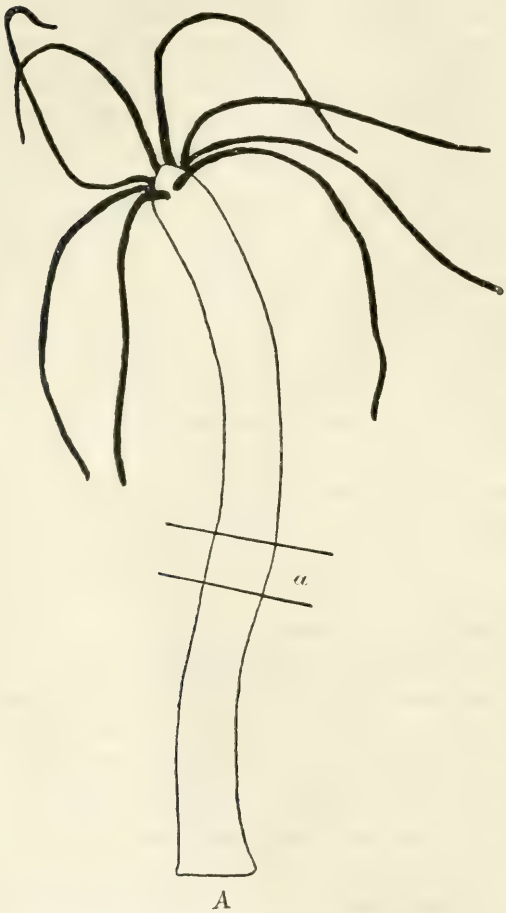
In the experiment in which only the head is cut off by an oblique cut the development of the new head can be more easily

followed. The new head is at first smaller than the one removed, Fig. 21, and lies to one side. As it enlarges we can see the old tissue of the middle line extending over towards the base of the new head. It looks as though a line of migration were being established between the median plane of the old part and that of the new head. As the head enlarges and is carried somewhat forward its middle line comes to correspond more nearly to that of the middle line of the body behind, and when the head has reached its full size it has reached a symmetrical position at the anterior end. It is evident that the smallness of the head, that first forms to one side, is in large part responsible for the lack of coincidence between its median plane and that of the old part.

My study of the regeneration of pieces of different shapes, and from different parts of the body, was undertaken in the hope of determining the factors at work during the change. One set of factors may determine in any given piece the position of the new head, tail and pharynx. These structures once being laid down the subsequent changes may be due to another set of factors or, it may be, to a continued action of the same kind. For instance the position of the new head in oblique pieces may be determined by the most anterior part of the old tissue. The head being once established by this factor, others may then come in and remould the old tissue to correspond to the position of the new parts. The remoulding that takes place in the old part seems to be due to a change in position of the old parts; and the change is of such a kind that the piece assumes more and more the proportions characteristic of each kind of worm. In the short cross-pieces as well as in the oblique pieces a very extensive transformation takes place, as great almost as takes place in a fragment of a protozoon, or of a piece of the body wall of hydra. I have made some experiments with rings cut from the body of hydra, that show how great a transformation may take place without the appearance of any new tissue as the accompanying figures show, Fig. 31. A short ring cut from the body of Hydra viridis, Fig. 31 *A*, closes its ends in the course of two hours, Fig. 31 a^1, a^2. Then for 24 to 48 hours it remains in the form of a ball or ellipsoid, Fig. 31 a^3. At the end of that time tentacles, usually only four, appear near one end of the piece, and the piece begins to elongate and fixes itself at one end, Fig. 31 a^4. There is no evidence of the formation of new tissue at the ends. The piece changes it shape as a whole, until after several days it has attained the proportions

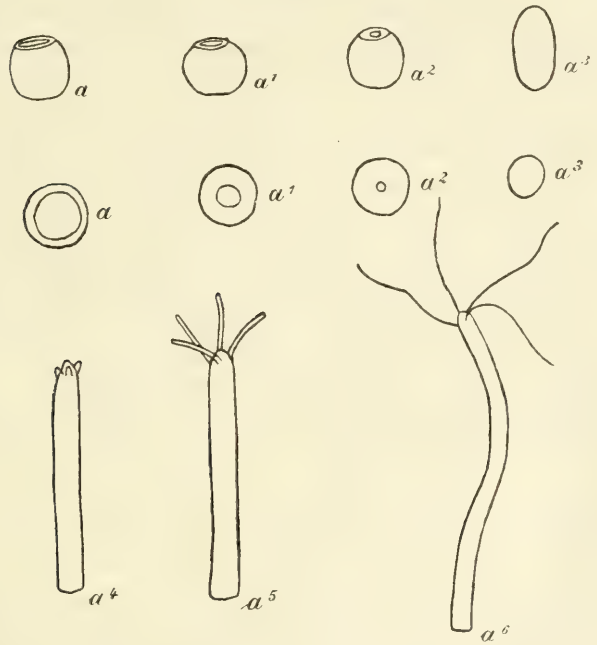
of the typical form, Fig. 31 a^5, a^6. The piece has formed a new hydra of the characteristic shape entirely out of the old material. A larger piece forms a larger hydra, a smaller piece a smaller hydra, and in both cases the characteristic form develops. At present I can think of this process only by assuming it to be the result of the physical form that any piece of the material of which the hydra is made will take under the conditions normal for that animal. In one sense this only restates the results, but the implication involved is, I think, an important one. At the time of separation of the piece it is a part only of a larger whole, and the peculiarity of the result consists in this, that the part changes its material throughout in such a way that it itself becomes a new whole. It has than reached a condition of physical equilibrium for that kind of organic material. I think of the changes as involving the piece as a whole without regard to cells or cell boundaries. The so-called formative forces I should identify with the physical changes in the material. Changes that are the result of the molecular (?) structure slowly assumed by the piece. They are the physical forces inherent in that kind of material. We know that many inorganic substances assume definite forms under given conditions, why then do we hesitate to call the changes in form of organic material the result of »formative« forces?

Fig. 31 *A*.



The answer is I think twofold. First because those who have referred the changes to formative forces have given the impression that, by the assumption, they intend to give a causal explanation of the phenomenon. This is in one sense a mistake, for we should have to assume as many such formative forces, in the present state of our knowledge, as there are different forms of organic beings. No one would question, however, the statement that the material of no two species is identical, and this difference in chemical composition would carry with it a

Fig. 31 a—a^6.



difference in the form that results from the material. All that is gained by referring the changes to formative factors is that we point out to what group of physical changes the phenomenon of morpholaxis belongs. It is a question of classification at best, and not a causal explanation, since we do not refer the phenomena to better known processes. The other reason that makes most biologists hesitate to refer the results to formative forces, in the sense defined above, is, I think, because they realize that by so doing they put the phenomena at once into a category that, in the present state of our knowledge, does not admit of further explanation, and they

hesitate to relinquish the problem in this way until they have exhausted all other means of solution.

The conception, that the morpholaxis is due to a tendency in a part separated from the rest to return to the typical form, i. e., to a state of equilibrium, is not, be it noted, a vitalistic one, but it is an idea strictly along physical lines. Driesch ('99) has attempted to prove that the analysis of a similar problem, viz., the typical proportions assumed by a part of the whole (embryo), obliges as to assume a vitalistic principle in nature. It seems to me, on the contrary that the problem can be safely rested on a physical conception even if we can not at present offer any causal explanation of the group of phenomena to which it belongs. I have no quarrel with those who prefer to use the words formative forces provided they define exactly what they mean, and grant that the terms include nothing more than a restatement of the phenomena. If we attempt, however, in the present state of our knowledge to account for the change of form itself we pass at once, as would be the case in any physical problem of the kind, beyond the present legitimate bounds of scientific research and land in metaphysics and vitalistic principles. Without, therefore, attempting to solve the ultimate problems involved in these changes of form, there are numerous questions in the study of regeneration that await our investigation. The factors that in each case influence the changes are, I believe, our more immediate problems, and are within reach of legitimate experimental work and speculation.

The position of the new head and tail on a piece cut obliquely.

In connection with the description of the experiments (page 89) I have attempted to give a brief analysis of the factors that determine the position of the new head in oblique pieces. A clue to the solution was found in the experiment in which a large part of the old head was removed by an oblique cut, Fig. 21, posterior to the eyes, but above the ear-lap of one side. The presence of the latter structure would determine, it was stated, one side of the new head. If it be asked on what evidence it is assumed that the new head would necessarily arise in connection with the old, rather than in the middle of the obliquely-cut side, I should reply, that the statement rests on the observed results of many experiments that show, that new organs arise from or near to the old ones of the same kind. It is more difficult to offer an explanation of the fact that the new

head does not extend over the entire length of the oblique surface, and its median plane correspond with that of the median plane of the old part, or at least stand at an angle with it. Other experiments have shown that we may regard the new material along the cut-edge as at first totipotent; at least a head may be formed from new material derived from any part of the exposed surface. The problem resolves itself, therefore, into accounting only for a sufficient stimulus at one point, and that in the case just cited is given by the presence of a part of the old head. This being determined the amount of new material that is taken up by the head will be regulated, in turn, by the nature of the material. The new head takes up only as much as its typical proportions demand — more or less according to the circumstances, but within certain limits for each set of conditions. Here we come to a point where, as before, our analysis comes to a halt, and we can only suppose the proportions to be fixed by the nature of the material.

In pieces cut off obliquely posterior to the head region (Fig. 14), or in posterior pieces with an oblique anterior cut-surface, the explanation of the position of the new head must depend upon the same, or similar factors. In these cases we must suppose in the absence of a definite part of the old head, that the position of the new head is determined by that part of the cut-edge that represents the most anterior part of the old tissue, and this is found at one side, and not in the middle line. If this could be conclusively demonstrated it would, I think, be a point of some importance on account of its connection with the so-called polarization phenomena that are so often referred to in connection with the problems of regeneration. As it is, I think, this interpretation is made extremely probable by the facts already described.

There is another possible way of looking at this phenomenon. If we cut off the piece more and more obliquely we are gradually approaching a longitudinal cut. From the side of a longitudinal piece lateral tissue regenerates. An oblique section may be thought of as combining the peculiarities of both cross and longitudinal pieces; the head appearing on the more anterior part of the new tissue, and, therefore, at one side, and the remainder of the new tissue forming the side of the piece. It seems to me, after some thought on the subject, that this interpretation is unsatisfactory since it begs the whole question by assuming that the compromise would take place in the way the result demands.

It is of some interest to consider the results of this experiment in connection with Sachs' ('80) view of formative substances that migrate in definite directions. In another paper (1900) I have examined this question in the light of Sachs' hypothesis, and while it must be granted that it gives us at least a formal explanation of the results, yet I have tried to show that the assumption of formative stuffs, and their migration in a given direction, is an interpretation that fails to explain other phenomena, particularly lateral regeneration, and in consequence it has seemed to me necessary to turn in other directions for a solution. The interpretation suggested in the preceding pages seems sufficient in itself to explain the lateral position of the head and tail in oblique pieces, and, I believe, it is a view that can be applied with success to other phenomena of regeneration.

Another problem connected with regeneration from an oblique surface is the position of the new head, Fig. 21, at right angles to the cut-surface. On pages 80—89 I have described the phenomenon, and need not recall here the main facts, since I shall soon take an opportunity of treating the entire question more adequately in connection with other experiments. The position of the head at one side of the piece does not have in itself, so far as I can see, anything to do with the oblique position of the new head. The amount of new material, and its form, at the time when the head is developing, seems to be the important factor in the result. In a case like that shown in Fig. 16 *b*, the new head that appears at the anterior end is derived in part only from material derived from the oblique surface, and in part from material at the side. The new head under these circumstances stands at the pointed end, occupying, as it were, the position of the resultant of the two tendencies. When two heads appear as in Fig. 17 *b*, each may stand at an angle with its nearest cut-surface.

Heteromorphosis.

The regeneration of a new head at the posterior cut-surface of the old head has been described on page 101, Fig. 30. This result is from a theoretical point of view extremely interesting, especially since it falls into line with similar results on other forms that have been recently obtained. I have been able to show in an earthworm (Allolobophora foetida) when the animal is cut in two posterior to the middle of the body, that from the anterior end of the posterior

or tail-piece there often regenerates a tail and not a head[1]). Bonnet has recorded a similar observation on Lumbriculus. In Allolobophora it has been shown by Miss A. P. Hazen ('99), working in my laboratory, that from the posterior end of a short anterior piece a new head may develop instead of a tail. The regeneration is extremely slow, and the small piece is kept alive by grafting it upon the anterior end of a larger piece. The result is due, I think, to the nature of the small piece, and not to its position on the anterior end of the larger piece. The result is, therefore, strictly parallel to that obtained with Planaria sp. ?.

Furthermore, in the tadpole Harrison has shown, that when a tail is grafted in a reverse position upon the body of another tadpole there grows out from its posterior end (formerly the anterior end), not a new tadpole, but a tail-like structure. Harrison after carefully discussing the result leaves it an open question as to whether the new structure is a tail, or only a suppressed and imperfect trunk or possibly a neomorphic growth. I have devised a new experiment by means of which the anterior growth can be more easily obtained. The tail of a young tadpole, — either before or after it leaves the membranes — is partially cut off at its base, a wedge shaped piece being removed from its dorsal side; the point of the wedge extending through the notochord to the aorta in the tail, or even ventral to that vessel. From the exposed anterior end of the tail there grows forward a tail like structure containing nerve-cord, notochord, and muscle-tissue. There can be little question that the new structure is a tail, and that it is the same structure obtained by Harrison in his grafting experiment. It is another case of heteromorphosis.

Herbst ('96) showed that when the eye of certain shrimps is cut off there sometimes regenerated an eye and sometimes an antenna. I showed ('98) later that when the tip of the eye-stalk is cut off an eye comes back and when the stalk is cut off at its base an antenna-like organ developed. Herbst ('99) independently obtained the same result, and thinks that the regeneration of the eye is dependent upon the presence of the old optic ganglion.

In some of the hydroids it has been shown by Loeb ('91), Bickford ('94), Driesch ('97) and myself ('98) that a head may appear on the proximal end of a piece removed from the stalk.

[1]) From a statement made by Spallanzani and referred to in a former ('99) paper I supposed that be had obtained a similar result, but Prof. Cattaneo has pointed out to me that Spallanzani meant something quite different.

Loeb in particular has shown in several other hydroids that heteromorphosis may be produced under the influence of external conditions.

In the higher forms in which external conditions do not have any influence in determining the kind of regeneration we turn to internal factors to see if we can discover what they are, and how they produce heteromorphosis. The obvious fact in regard to the heteromorphic regeneration in the planarian, earthworm and tadpole in that like produces like, and that the result is in some way connected with particular parts of the body. If the cut is made through the head a heteromorphic head is produced, if through the tail a tail regenerates.

What factors can we suppose bring about this result? On the Bonnet-Sachs' hypothesis of specific stuffs the explanation is at hand. But for reasons given elsewhere (1900) I do not believe we are justified in assuming the presence of specific stuffs of this kind in the cases under discussion. Moreover on both Bonnet's and Sachs' views these specific stuffs are supposed to be made in other parts of the body than in the regions in which they act, i. e., the head-forming stuffs are made in the body, and transported forward, and tail forming stuffs are made in the body and carried posteriorly into the tail. On this view we have no grounds for supposing that these stuffs are present in especially large amounts in the head and tail at the time of the operation. Furthermore in the earthworm the heteromorphic regeneration may not take place until several months after the operation, and during that time such stuff would in all likelihood have disappeared.

Can we account for the results on the assumption of the specification of the tissues. This view differs from the former in this, that it supposes the physical or chemical structure or material of the old part to be carried over into the new, and there influence the kind of regeneration that takes place. The specification might be due to specific substances contained in the part or to the specific structure of the living part itself. Let us ask first how far we can explain the ordinary process of regeneration as the outcome of specification of the tissues? For example, the regeneration of the limb of a Salamander! At whatever level the limb is cut off as much as is removed comes back. Can we explain this as due to the difference in the specification of the material? At each level the kind of new material that develops at the cut-surface would be different; but the new material at each level give rises to the whole

of the missing part, and unless we fall back on a praeformation hypothesis, as Weismann has done, the hypothesis of specification is in itself insufficient to explain the result. In this process there can be, I think, little doubt that the relation of the new part to the old is the determining factor that regulates the kind of result at each level. In other words, on my view, the new material is practically the same at every level — and the new material is omnipotent as far as concerns the formation of a new limb. The factor that determines how much of the new limb is to form in this case is the physical, structural relation of the old and new parts. In other words the specification of the tissue does not explain the principle result. How far a specification may account for the production of ectoderm by ectoderm, bone by bone, muscle by muscle is a question that need not be here considered.

In the case of the ordinary regeneration of a head in the earthworm, where a head of only five segments comes back when an anterior end containing a greater number of segments has been removed, the relation of the old to the new part plays a less obvious part. In this case, as I have tried to show (1900), the result is dependent upon the amount of new material that is first formed.

If the preceding instances of ordinary regeneration can be accounted for without assuming a problematical specification of the tissues it does not of course follow that the hypothesis of specification may not account for the production of heteromorphic structures, yet, I think, we should be cautious before applying this explanation. On the otherhand the heteromorphosis is so obviously connected with the nature of the part in which it appears that, for the present at least, I can see no alternative except to suppose that there is something in the structure or composition of the head and tail, that may act as a determining factor in the production of heteromorphic regeneration.

Summary.

1. The study of change in form (morpholaxis) of pieces of different shapes is the principle object of this contribution.

When a planarian is cut in two in front of (Fig. 1) or just behind the pharynx (Fig. 2) the presence of the oral, or of the genital openings on the posterior pieces give definitive landmarks by means of which the subsequent changes may be followed. A new head

develops on the posterior pieces out of the new material that first appears (Fig. 1 *F*). Later the entire piece becomes narrower and longer, the new head being carried further forward by the development of new material in the »intermediate« region just behind the head (Fig. 2 *E*—*G*). In Planaria sp.? the new pharynx in a posterior piece cut off behind the old pharynx (Fig. 2 *y*—*y*) develops just behind the new head (Fig. 2 *F*). The region between the head and pharynx grows longer until the two structures are widely separated. In Planaria maculata the new pharynx appears in the anterior part of the old tissue. The presence of the genital pore in the more anterior part does not even delay the development of the new pharynx.

2. Short cross-pieces (Fig. 3 *b*) become narrower than do longer cross-pieces (Fig. 3 *c*) as a new worm develops.

3. Experiments on Bipalium (Fig. 4) show that the elongation of a cross-piece not only takes place at the ends, where new material is formed, but also in the old parts.

4. If a worm is cut in two longitudinally (Fig. 5 *d*) each half produces new material at the side, and the new pharynx appears between the old, and the new parts. If the new worm is not fed the development of a symmetrical form goes on, after the first production of new material, very slowly and the new part increases at the expense of the old, Fig. 5 *d'*. The worm, as a whole, may decrease in size through lack of food. If the half is fed the new side grows more rapidly, and a symmetrical form is soon produced, Fig. 6 *d'*. Pieces half the breadth of the worm, but less than the entire length (Fig. 5 *a*, *b*, *c*), produce less new material at the side, and form smaller worms (Fig. 5 *a'*, *b'*, *c'*).

5. Narrower pieces from the side (Fig. 8 *c*) also produce, after a time, symmetrical worms. In several pieces of this kind one eye developed before the other (Fig. 8 *a*). The right-angled forms (Fig. 7 *b*), that often develop from small pieces, are not the result of the shape of the pieces, but seem to be due to the small amount of new tissue produced.

6. Short cross-pieces were divided unequally into two parts, Fig. 10 *a*, *b*. Very little new tissue forms at the sides of these pieces, and right-angled forms are often produced in the smaller pieces.

7. Short cross-pieces were allowed to develop for a few days, and then cut in two longitudinally before the new material had produced a new head, Fig. 11. The half-pieces produced at first only one eye; the other eye coming in later, Fig. 12 *a* *a*^{1}. The

new material, it would seem, continues to produce its half-structure while at the same time the missing half is regenerated.

8. Pieces cut off by two parallel oblique cuts, Fig. 14 *A*, produce a new head at one side of the anterior oblique cut-surface and a new tail at one side of the posterior oblique surface (Fig. 14 *a*—a^3). The new pharynx develops in the old middle line near the posterior edge. The greater part of the new material along the anterior and posterior oblique edges produces the sides of the new worm. The old tissue is worked over and a symmetrical worm is formed.

Pieces cut out by two oblique cuts as shown in Fig. 15 *A* produce a new head and tail at one side as before, but now on the same side, Fig. 15 *a*. The new pharynx is as before in the old middle line. Subsequent changes produce a symmetrical worm.

Pieces cut out as shown in Fig. 16 *A*, *a*, *b*, also produce new heads at the most anterior region of the old part; in the piece, *a*, at the right outer side of the anterior oblique edge, and in *b* at the right inner side of the anterior oblique edge, or at the anterior angle. Sometimes two heads are produced in *b*.

If a worm is cut in two as shown in Fig. 18 *A*, the posterior piece produces a new head at the apex if the old part.

If a worm is cut in two as shown in Fig. 19 *A*. usually one head is produced in the angle, because the sides shorten and the new tissue is continuous across the middle line, Fig. 19 *a*. But if, after the first operation, the posterior piece is split down the middle line for a short distance, as indicated in Fig. 19 *B*, two heads may be produced one on the anterior outer edge of each half, Fig. 19 *b*. Double tails may also be produced by oblique cuts (Fig. 20 *A*).

If a portion of the head is cut off by an oblique cut (Fig. 21 *A*) the new head is at first smaller than the one removed. The development of the new head is shown in Fig. 21 *a*—a^4. If the oblique cut is further back in the head-region as shown in Fig. 21 *B* the new head develops as shown in Fig. 21 *b*—b^4.

An attempt is made on pages 88—89 to give an analysis of the process of formation of new head and tail at the side of an oblique edge.

9. In another experiment the anterior end was first cut squarely off, Fig. 22 *A*, and then by two parallel longitudinal cuts a small median piece, *x*, was cut out. The sides came together and a new head developed on the anterior end, Fig. 22 *a*. The absence of the middle part neither delays the production of the new head, nor produces any change from the normal.

10. Oblique and triangular pieces from the head region (Fig. 23 *A*) may produce new worms.

11. If cross-cuts are made at two levels as shown in Fig. 24 *A*, so that there are two anterior exposures at different levels only one head is usually produced Fig. 24 *b*. In one case two heads developed; one on each exposed edge, Fig. 25 *b*.

12. Double headed and double tailed forms are produced by partial longitudinal splitting, as is known. If a worm is split from behind forward as far as the base of the new head one or two new heads may appear in the angle between the halves, Fig. 27 *a*, *a*^{1}. If a worm Planaria sp.? is split still further forward (Fig. 28), each half of the old head may complete itself and two worms united by a narrow anterior end be the result.

In several cases worms with a bifid posterior part were operated upon as indicated in Fig. 26. The results are described on page 96.

13. Below a minimum size new worms are not produced. Very small half-pieces through the eye, Fig. 29, may make new worms.

14. If the head of Planaria sp.? be cut off through the region just behind the eyes the old head produces, in a large percentage of cases, another head at its posterior end, Fig. 30. This is a positive case of heteromorphosis.

15. The problem of morpholaxis, the interpretation of the lateral position of the new head and tail in oblique cuts, and the origin of heteromorphosis are discussed in the Theoretical Part of the paper, in connection with the so-called formative forces.

Bryn Mawr, Penn., April 9 1900.

Zusammenfassung.

1. Das Studium der Gestaltveränderungen (Morpholaxis) von verschieden gestalteten Stücken ist der Hauptgegenstand dieses Beitrages.

Wenn man eine Planarie vor (Fig. 1) oder kurz hinter (Fig. 2) dem Pharynx in zwei Theile zerschneidet, ergiebt die Gegenwart der Mund- bezw. der Genitalöffnung bestimmte Merkzeichen, durch welche die Verfolgung der späteren Veränderungen ermöglicht wird. Ein neuer Kopf entwickelt sich an dem hinteren Stücke auf Kosten des neuen Bildungsmaterials, das zuerst erscheint (Fig. 1 *F*). Später wird das ganze Stück schmäler und länger, indem der neue Kopf immer weiter nach vorn geschoben wird, weil sich neue Massen in der »Zwischen«- (intermediate) Region ausbilden, unmittelbar hinter dem neuen Kopf (Fig. 2 *E*—*G*). Bei Planaria sp.? entwickelt sich der neue Pharynx in einem hinter dem alten Pharynx abgeschnittenen hinteren Stücke (Fig. 2 *y* *y*^{1} unmittelbar

hinter dem neuen Kopf (Fig. 2 *F*). Das Gebiet zwischen Kopf und Pharynx verlängert sich bis zu weiter Trennung der beiden Gebilde. Bei Planaria maculata erscheint der neue Pharynx im vorderen Theile des alten Gewebes. Die Gegenwart des Genitalporus im vorderen Stück bewirkt nicht einmal eine Verzögerung der Pharynxneubildung.

2. Kurze Querabschnitte (Fig. 3 *a*) werden beim Auswachsen zum neuen Wurm schmäler als längere solche Stücke.

3. Experimente mit Bipalium (Fig. 4) zeigen, dass die Verlängerung eines Querabschnittsstücks nicht nur an den Enden erfolgt, wo neues Material sich ausbildet, sondern auch in den alten Theilen.

4. Wird ein Wurm längs getheilt (Fig. 5 *d*), bildet jede Hälfte seitwärts neues Material an und der neue Pharynx erscheint zwischen dem alten und den neugebildeten Theilen. Wird der neue Wurm nicht gefüttert, so erfolgt die Entwickelung zur symmetrischen Form nach der ersten Ausbildung neuen Materials sehr langsam und der neue Theil wächst auf Kosten des alten heran (Fig. 5 *d*). Durch den Futtermangel kann sich der Wurm als Ganzes verkleinern. Werden die Hälften dagegen gefüttert, so wächst die neue Seite schneller heran und bald ist eine symmetrische Form entstanden (Fig. 6 *d'*). Stücke von halber Wurmbreite aber weniger als der ganzen Wurmlänge (Fig. 5 *a*, *b*, *c*) entwickeln weniger Material an der Seite und bilden kleinere Würmer (Fig. 5 *a'*, *b'*, *c'*).

5. Noch schmälere Stücke von der Seite (Fig. 8 *c*) lassen gleichfalls nach einiger Zeit symmetrische Würmer entstehen. Bei manchen derartigen Stücken entwickelte sich das eine Auge vor dem anderen (Fig. 8 *a*). Die rechtwinkeligen Formen (Fig. 7 *b*), die sich oft aus kleinen Stücken entwickeln, sind nicht durch die Gestalt der Stücke verursacht, sondern scheinen der quantitativ geringen Gewebsneubildung ihre Entstehung zu verdanken.

6. Kurze Querabschnitte werden in zwei ungleiche Theile getheilt (Fig. 10 *a*, *b*). An den Seiten dieser Stücke bildet sich sehr wenig neues Gewebe und rechtwinkelige Formen entstehen oft aus den kleineren Stücken.

7. Kurze Querabschnitte ließ ich einige Tage sich regeneriren und theilte sie dann der Länge nach, bevor das neue Material einen neuen Kopf gebildet hatte (Fig. 11). Die halbirten Stücke entwickelten zunächst nur ein Auge, das andere kam erst später hinein (Fig. 12 *a*—*a*⁴). Das neue Material fuhr allem Anschein nach fort, seine halbirten Gebilde zu entwickeln, während gleichzeitig die verlorene Hälfte regenerirt wurde.

8. Durch zwei Schrägschnitte erhaltene Stücke (Fig. 14 *A*) bilden einen neuen Kopf an der einen Seite der vorderen Schrägschnittfläche und einen neuen Schwanz an der einen Seite der hinteren (Fig. 14 *a*—*a*³). Der neue Pharynx entwickelt sich in der alten Mittellinie, dem hinteren Rande näher. Der größere Theil der Neubildung entlang der vorderen und hinteren Schrägschnittfläche bildet die Seiten des neuen Wurmes. Das alte Gewebe wird umgearbeitet und ein symmetrischer Wurm gebildet.

Durch zwei Schrägschnitte nach Fig. 15 *A* erhaltene Stücke entwickeln neuen Kopf und Schwanz an einer Seite, wie vorhin, aber jetzt an derselben Seite (Fig. 15 *a*). Der neue Pharynx liegt wie vorher in der Mittellinie. Spätere Veränderungen lassen einen symmetrischen Wurm entstehen.

Stücke nach Fig. 16 *A*, *a*, *b* ausgeschnitten produciren gleichfalls neue Köpfe an den vordersten Enden der alten Theile; an der rechten Außenseite des vorderen schrägen Randes des Stückes *a* und an der rechten Innenseite gleichen Randes des Stückes *b*, oder am vorderen Winkel.

Beim Zerschneiden eines Wurmes nach Fig. 18 *A* bildet das hintere Theilstück ein Haupt an der vordersten Ecke des alten Theils.

Beim Zerschneiden nach Fig. 19 *A* entsteht gewöhnlich ein Kopf an der Ecke, weil die Seiten sich verkürzen und das neue Gewebe quer zur Mittellinie kontinuirlich zusammenhängt (Fig. 19 *a*). Wenn aber nach der ersten Operation das hintere Stück in der Mittellinie eine kleine Strecke weit gespalten wird (vgl. Fig. 19 *B*), können zwei Köpfe am vorderen Außenrande jeder Hälfte entstehen (Fig. 19 *b*). Doppelte Schwänze (Fig. 20 *A*) können auch durch Schrägschnitte erzielt werden.

Wird ein Theil des Kopfes durch einen Schrägschnitt entfernt (Fig. 21 *A*), so ist der neugebildete Theil zunächst kleiner als der entfernte. Die Entwickelung des neuen Kopfes zeigt Fig. 21 *a*—a^4. Fällt der Schrägschnitt in der Kopfgegend weiter nach vorn (Fig. 21 *B*), so entwickelt sich der neue Kopf wie Fig. 21 *b*—b^4 zeigt.

Auf pag. 89 habe ich versucht, die Kopf- und Schwanzbildung an der Kante eines schrägen Schnittes zu analysiren.

9. Bei einem anderen Versuche wurde das Vorderende zunächst quer abgeschnitten (Fig. 22 *A*) und darauf durch zwei parallele Längsschnitte ein schmales Mittelstück (*x*) herausgetrennt. Die Seiten schlossen sich zusammen und am Vorderende entstand ein neuer Kopf (Fig. 22 *a*). Die Abwesenheit des Mittelstücks verzögert weder die Kopfneubildung noch bewirkt sie sonst Abweichungen vom normalen Verhalten.

10. Schräge und dreieckige Stücke der Kopfgegend können neue Würmer erzeugen (Fig. 23 *A*).

11. Werden Querschnitte in zwei Höhen (Fig. 24 *A*) angelegt, so dass dann zwei vordere Wundränder in verschiedener Höhe vorhanden sind, so wird gewöhnlich nur ein Haupt gebildet (Fig. 24 *b*). In einem Falle entwickelten sich zwei Köpfe, einer an jedem vorstehenden Rande (Fig. 25 *b*).

12. Doppelköpfige und doppeltgeschwänzte Formen entstehen bekanntlich durch theilweise Längsspaltung. Spaltet man einen Wurm von hinten so weit ab, als die Basis des neuen Kopfes reicht, so können ein oder zwei neue Köpfe in dem Winkel zwischen den Hälften entstehen.

Spaltet man einen Wurm (Planaria sp.?) noch weiter nach vorn (Fig. 28), so kann sich jede Hälfte des alten Kopfes ergänzen und es resultiren dann zwei mit dem Vorderende vereinigte Würmer. In normalen Fällen wurde an den Würmern mit zwiegespaltenem Hinterende operirt, wie in Fig. 26 angegeben. Die Resultate sind pag. 96 beschrieben.

13. Unterhalb einer gewissen Minimalgröße (der Stücke) werden keine neuen Würmer mehr hervorgebracht; doch können sehr kleine halbseitige Stücke im Gebiet des Auges solche erzeugen.

14. Wenn der Kopf von Planaria sp.? im Gebiete unmittelbar hinter den Augen abgeschnitten wird, so entwickelt sich bei einem großen Procentsatz der Fälle hinten an dem alten Kopf ein anderer neuer (Fig. 30). Das ist ein positiver Fall von Heteromorphosis.

15. Das Problem der Morpholaxis, die Auslegung der seitlichen Stellung des neuen Kopfes und Schwanzes in Schrägausschnitten und der Ursprung der Heteromorphosis werden im theoretischen Theil der Arbeit erörtert, im Zusammenhang mit den sog. gestaltenden Kräften.

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Regeneration in Teleosts.

By

T. H. Morgan.

With 14 figures in text.

Eingegangen am 27. April 1900.

An examination, that I made, of fish that had just been caught and brought into the laboratory at Woods Holl showed that the tail had often been injured and had in many cases begun to regenerate. In discussing the question with Prof. H. C. Bumpus he told me that he had experimented with Fundulus and had found that the tail readily regenerates. I began, therefore, my experiments on this form

Fig. 1.

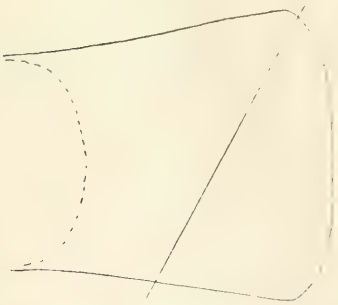
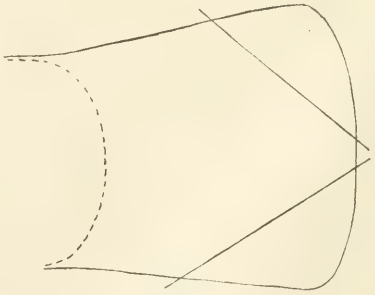


Fig. 2.



(Fundulus heteroclitus). Cutting off the tail squarely at different levels I saw in a few days the formation of new material along the edge. This new part grew rapidly, and in the course of two weeks or longer, according to the amount removed, the tail regained its former size.

At this time I was studying the regeneration of planarians, and was greatly interested in regeneration from an obliquely cut surface.

I therefore tried the same experiment with the tail of Fundulus. The tail was cut off obliquely in several fish as shown in Fig. 1 (July 25). At the same time the tails of three other fish were cut

Fig. 3.

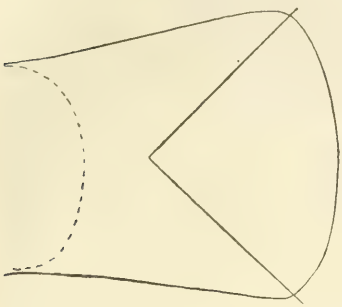
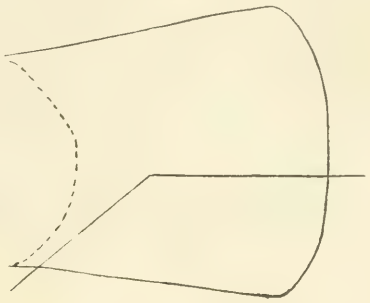


Fig. 4.



off by two oblique cuts as shown in Fig. 2; and of three others by two oblique cuts as shown in Fig. 3. In three other fish the tail was first split longitudinally, and then the lower half cut off by an

Fig. 1 *a*.

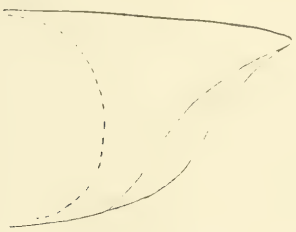
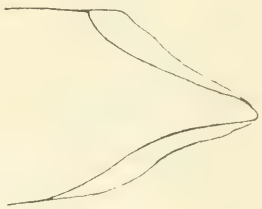
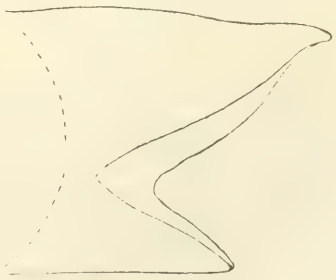


Fig. 2 *a*.



oblique cut, Fig. 4. Twelve days after the operation the fish were killed and one tail from each of the first three experiments is drawn[1]) in Figs. 1 *a*, 2 *a*, 3 *a*. In the first series, Fig. 1 *a*, the new material is broader at the lower part of the cut-edge than at the upper. In the second series, Fig. 2 *a*, the new material is broadest at the upper end of the upper oblique cut,

Fig. 3 *a*.



[1]) The figures that indicate the kind of operation, as Figs. 1, 2, 3 for example, are intended to represent the position of the cut on the living tail. The figures that represent the new, regenerated part, as Figs. 1 *a*, 2 *a*, 3 *a* for

and at the lowest end of the lower cut. In this case also the greatest amount of new material has developed at that part of the edge that is nearer the base of the tail. In the third series, Fig. 3 *a*, the new material is widest at the inner angle, where the oblique-cuts meet, i. e., again on that part of the oblique surface that lies nearer to the base of the tail. In the fourth series new material had developed at the exposed edge of the oblique part of the cut, but almost no new material was present along the side of the longitudinal cut.

Meanwhile another experiment had been started. The tail of one fish was cut squarely across at about the middle, Fig. 5, and

Fig. 5.

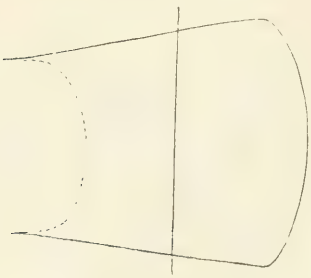


Fig. 5 *A*.

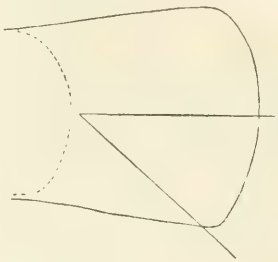


Fig. 5 *a*.

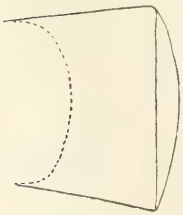
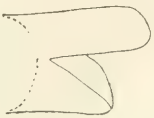


Fig. 5 *A*, *a*.



the tail of another was cut as shown in Fig. 5 *A*. In the former the new part, after nine days was broader at the middle than at the sides, Fig. 5 *a*. In other words it has assumed the characteristic, rounded form of the tail of Fundulus, although the new part had by no means replaced as much as had been lost. In this experiment the cut was made strictly across the tail, yet after a time the middle

example, are camera drawings of preserved material. The differences in size of the two kinds of drawings are in part the result of shrinkage, due to the preservatives in the latter series, and also due to fish of different sizes being used.

part grew faster than the sides, and as a result the new part assumed a rounded form. In the other experiment the new material grew faster at the inner part of the oblique surface and very little new material formed at the edge of the longitudinal cut, Fig. 5 *A*, *a*.

The more rapid growth that took place in all these experiments was in that part of the oblique cut that lies nearer the base of the tail. The results suggested that the new part might receive better nourishment at the base of the tail than further out, hence its more rapid growth. This view would also account for the graded rate of

Fig. 6 *a*.

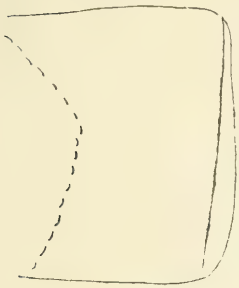


Fig. 6 *b*.

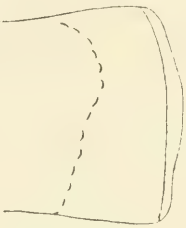


Fig. 6 *c*.

Fig. 6 *d*.

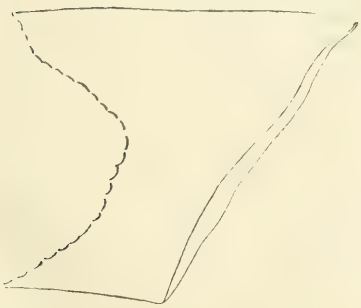
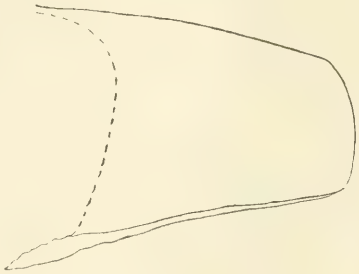


Fig. 6 *e*.



growth from the inner to the outer part of the oblique cut. I attempted to test this interpretation of the process by cutting off the tail in some fish near the base, and in others near the outer part of the tail. The fish were kept under similar conditions, and the relative rate of growth examined from day to day. In an experiment begun Aug. 2 the tails of two large fish were cut-off, one near the outer end, and one near the base. At the same time the same operation was carried out on two smaller fish. Seven days later the amount of new material in each seemed to be about the same. Four days

later there was no more new tissue on the shorter tail than on the longer one. The experiment was repeated on Aug. 19, when one tail was cut off near the base, one near the middle, and one near the outer part of the tail (the latter a little obliquely). The fish were killed on Aug. 25. The amount of new tissue is not obviously greater in the shorter, Fig. 6 *c*, than in the median tail, Fig. 6 *b*, or in the longer one, Fig. 6 *a*. The tails of two other fish were cut off obliquely at the same time. One had only the lower angle of the tail cut off, Fig. 6 *e*; the other had the entire tail cut across obliquely, Fig. 6 *d*. Six days later the tails appeared as drawn in Figs. 6 *e* and 6 *d*. The amount of new material at the base is about, the same as at the maximum points in the tails cut across, Fig. 6 *a*, *b*, *c*; but nearer the outer parts of the tail the amount of new tissue is distinctly less than in Figs. 6 *a* and 6 *b*, that are cross-sections at corresponding levels. This comparison shows that the view that the more rapid growth is only due to the level at which the cut is made is insufficient to explain the phenomena.

Fig. 7.

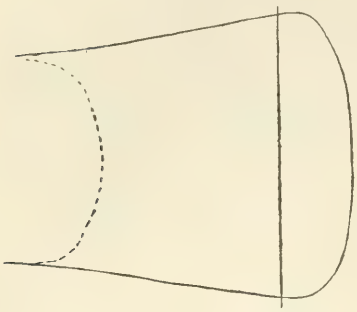


Fig. 8.

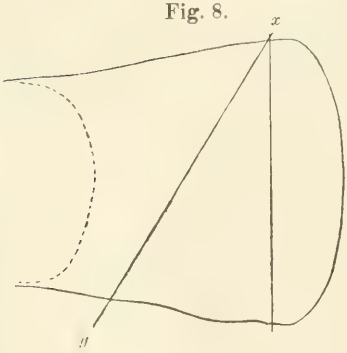
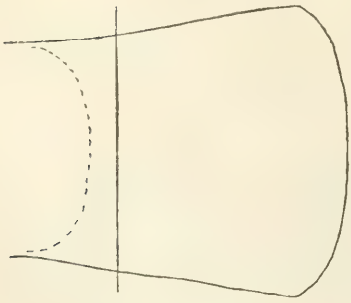


Fig. 9.



The results of the experiment on tails cut obliquely may be stated in a teleological way as follows: The more rapid growth takes place in those regions where more new tissue is needed to complete the entire structure. The general bearing of this statement will be further discussed later.

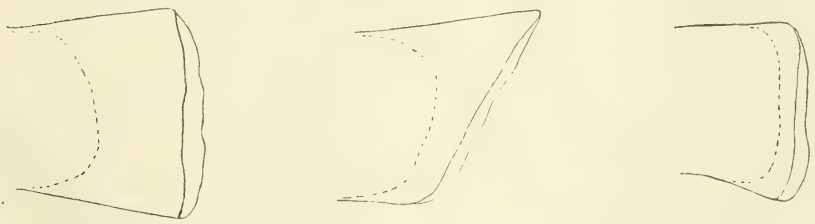
In another way I attempted to determine the meaning of the relation of the amount of growth from oblique surfaces. The tails

of two fish were cut squarely off as shown in Figs. 7 and 8; then one of these, Fig. 8, was cut off obliquely. The upper edge of the oblique cut (Fig. 8) lies at the same level as the cross-cut of the other tail (Fig. 7). The tail of a third fish, Fig. 9, was cut squarely off at its base, — at the level of the inner edge, *y*, of the oblique cut (Fig. 8). If the rate of growth over the oblique surface is determined by the nearness of the cut-surface at each point to the base

Fig. 7 *a*. Fig. 8 *a*. Fig. 9 *a*.

of the tail, then we should expect the new tissue at *x* in Fig. 8 to grow as fast as that over the squarely cut-surface of Fig. 7; and we should expect the growth at *y* in Fig. 8 to be the same as that over the cut-surface of Fig. 9. After eight days the fish were killed and their tails were drawn (Figs. 7 *a*, 8 *a*, 9 *a*). Comparing the rate of growth in the obliquely-cut tail with the other two we see at a glance it is narrower at the upper part than the broadest part of the

Fig. 7 *a'*. Fig. 8 *a'*. Fig. 9 *a'*.

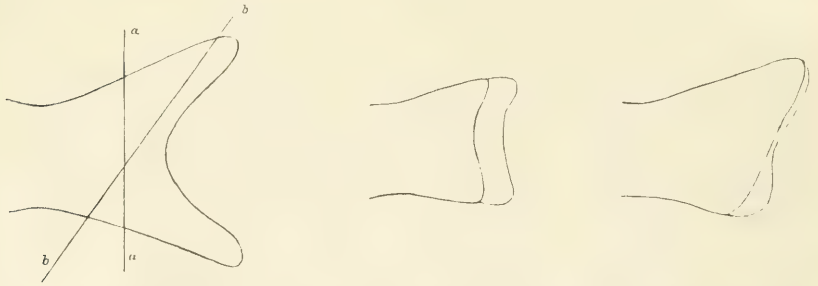


new tissue in the tail cut across (Fig. 7), and that the growth at the lower edge, *y*, is even greater than that in the tail cut across at the same level (Fig. 9 *a*). In another experiment of the same sort on somewhat larger fish the results two days later are represented in Figs. 7 *a'*, 8 *a'*, 9 *a'*. Exactly the same results were obtained except that the growth at *y* in Fig. 8 *a'* is more nearly like that in the shorter tail, Fig. 9 *a'*. The difference in this regard that was found in the preceding experiment is probably due to the fact, that in that experiment the shorter tail (Fig. 9 *a*), was cut too near the base:

passing, in fact, through the scales at the base of the tail. The latter experiment is, therefore, more satisfactory.

These results show, I think, conclusively, that the more rapid growth at the inner part of the cut-edge in an oblique cut can not be explained by the nearness of the cut to the base of the tail as compared with the outer parts of the same cut-edge. The experiments also show that it is not so much the more rapid growth at y as the retardation of the growth at x that is in need of explanation; for the latter, at x, is less than that at the edge of a cross-section at the same level, while the growth at y is about the same as that at the same level in a cross-section.

Fig. 10. Fig. 10 *A*. Fig. 10 *B*.

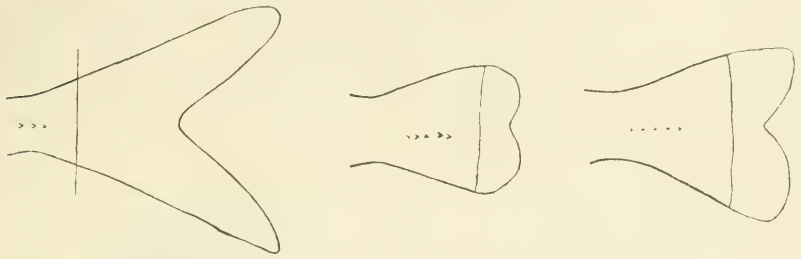


In seemed to me that this problem of the rate of growth might be made clearer, if, instead of using a form with a rounded tail like Fundulus, I should use a fish with a swallow-tail. Through the courtesy of Prof. H. C. Bumpus, in charge of the U. S. Fish Commission Laboratory at Woods Holl I was able to experiment with some of the fish in the aquaria of the station. I used first some young scup (Stenopus chrysops). The tail of this fish is distinctly bi-lobed, Fig. 10. In the first experiment (Aug. 9) the tails of five fish were cut squarely off near the base, Fig. 10 *a*—*a*; and the tails of five others were cut off obliquely as shown in Fig. 10 *b*—*b*. Seven days later new tissue was present along the cut-edge of all the tails, and it seemed at this time to be equally broad along its entire length. Two days later one could see that in the tails cut squarely off the outer parts were a little broader than the middle part, Fig. 10 *A*; and in the tails cut off obliquely the lower side of the new part was a little broader than the upper part, Fig. 10 *B*. In other words the new part was assuming the form of the old tail, although not as much new material had as yet developed as had been removed.

The most important fact shown by this experiment is that the regions of more rapid growth are directly connected with the development of the typical form of the tail, and not with the level at which the section has been made.

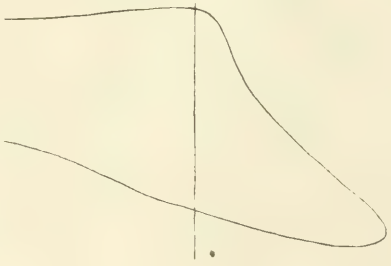
I repeated the experiment on some half-grown mackerel scad (Decapterus macrella). The tail of this fish is more bifid Fig. 11)

Fig. 11. Fig. 11 *A*. Fig. 11 *A'*.



than that of the scup. Four days after the operation the new material over the cut-edge was the same breadth along the entire edge, and it remained so for three days more, and then it began to grow faster at the upper and the lower parts. The day following it was distinctly bifid, Fig. 11 *A*, and continued to become more and more so (Fig. 11 *A'*, four days older).

Fig. 12.



The tail of the king-fish (Menticirrhus saxatilis) has the form shown in Fig. 12. The tails of two young fish were cut off by a cross-cut as shown in Fig. 12. Seven days later the new material was equally well developed alone the entire cut-edge in one of the two individuals; and in the other the new material was a little broader over the lower side. Four days later this fish showed a distinctly longer part on the lower side; the other tail had grown irregularly, due perhaps to injury. The same experiment repeated on another individual showed also after a time more rapid growth on the lower side.

The results of these experiments on the scup and the mackerel scad fall into line with those on Fundulus, and show that the more

rapid growth takes place where more tissue is to be formed (teleological view), or better, the new material assumes the form of the old part before the new material has reached its full growth. The development of the typical form is brought about by more rapid growth taking place in one (or more) parts than in another.

Oblique position of the new part on an oblique surface.

The new fin-rays can be seen at a very early stage in the new part, and on closer examination it will be found that in tails cut off obliquely the new rays make an angle with the old ones as a result of their position at right angles to the cut-surface. In Fig. 13 the

Fig. 13.

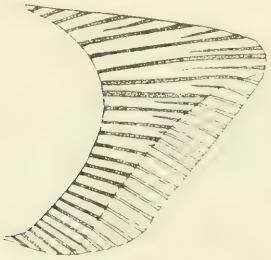
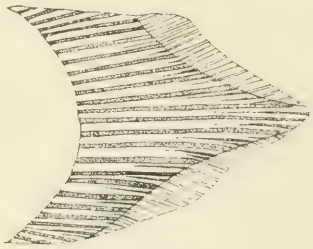


Fig. 14.



position of the new rays is shown in a tail cut off by one oblique-cut. In Fig. 14 the new rays in both the upper and lower new portions make an angle with the old ones. When the tail is cut as in Fig. 2 we would expect to find the rays standing at an angle with the old ones but turning inwards in this case, I have not been able to convince myself entirely that they do so. After cross-section of the tail the new rays continue in the same line with the old ones.

There can be no doubt that the position of the new rays, in tails cut obliquely, at right-angles to the cut-surface, is the same phenomenon that has been observed in several other forms. Barfurth [1] in 1891 showed that if the tail of a tadpole is cut off by an oblique cut the new tail will stand at right-angles to the cut-surface. He also showed that in later stages the tail swings back into place. Barfurth attempted to show by a series of ingenious experiments that this righting of the tail is a result of its being

[1] Barfurth, D., Versuche zur funktionellen Anpassung. Archiv f. mikr. Anat. 37. '91.

used as a swimming organ; for if the tadpoles were kept in shallow water, where they could not use their tails, the new tail did not in most cases swing back into line. The demonstration is not altogether satisfactory, as BARFURTH himself pointed out, since some of the tails did become straight under the conditions just described. In addition to the use of the tail as a factor in its straightening BARFURTH introduces other factors, »regulatorische Fähigkeiten«.

In 1895 I showed[1]) that in the earthworm the new head that develops from an oblique (lateral) surface stands at right-angles to the cut-surface. HESCHELER[2]) ('96) independently obtained the same result. DRIESCH[3]) has shown ('96) in Tubularia that the new tentacles that are laid down in the old part form an oblique row if the piece has been cut off obliquely. Whether this phenomenon belongs in the same category as the other cases described above may be open to question. KING[4]) has shown that in the regeneration of an arm of the star-fish from an oblique surface the new arm stands at right-angles to the cut-surface.

In planarians I have shown[5]) that when the anterior end is removed by an oblique-cut the new head, developing on the posterior piece, stands at an angle to the cut-surface. In these animals the new part appears not only at right-angles to the cut-surface, but it lies on only one side of the oblique-edge. In all the other forms described above the new part occupies the entire cut-surface. In these same planarians the new tail that develops on an oblique surface also stands at right-angles to that surface. The way in which the new head develops in planarians has given me a clue, I believe, to the interpretation of the phenomenon. The new head forms out of the new material long before it has increased sufficiently in amount to represent as much as was removed. The new head forms symmetrically in the new material, and since at this time the material has developed at about the same rate along the entire oblique surface, or at least in the region where the head appears, the median

[1]) MORGAN, T. H., A Study of Metamerism. J. J. M. S. 37. '95.

[2]) HESCHELER, K., Über Regenerationsvorgänge bei Lumbriciden. Jenaische Zeitschr. '96.

[3]) DRIESCH, H., Zur Analyse der Reparationsbedingungen bei Tubularia. Vierteljahrsschr. d. Nat. Ges. Zürich. '96.

[4]) KING, H. D., Regeneration in Asterias vulgaris. Archiv f. Entwickelungsmechanik. '98.

[5]) MORGAN, T. H., Experimental Studies of the Regeneration of Planaria maculata. Archiv f. Entwickelungsmech. '98.

line of the new head must stand at right-angles to the surface, if a symmetrical head is to form in this material. The objection that may be made to this statement is to the assumption that a new head must form symmetrically in the new part. Why this should happen I can not, of course, pretend to say, but the statement rests on the observed fact that the new head does form in this way. As I have tried to show in another paper the new material along the cut-edge in these planarians may be thought of as totipotent, or at least a new head may develop at any point along the obliquely cut surface. The factor that determines the position of the new head is the most anterior part of the old tissue. The position of the head being fixed the new head forms symmetrically in the new material that has appeared, and, in consequence, stands at right-angles to the cut-surface.

The development of a new head at right-angles to the cut-surface is shown in its most extreme form in pieces of the planarian cut longitudinally from the side [1]). The new head stands, at first, at right-angles to the cut-surface, and therefore at right-angles to the long axis of the old part. The new head soon grows faster on one side, and in consequence swings around into an anterior position.

Can we apply this same interpretation to the regeneration from an oblique surface in the tail of the fish?

The simplest case is that of regeneration from one oblique cut (Figs. 1 and 13). My observations go to show that at first the amount of new material along the cut-edge is equal at every point. From this new material a tail may now be thought of as forming with the middle axis of the new tail in the middle of the new material. It will stand, therefore, at right-angles to the cut-surface, and make an angle with the middle ray of the old tail. The end of the middle ray of the old tail will determine the point at the base of the new tail from which the middle ray is to develop, and the position of the new middle ray may be determined by joining the end of the middle ray of the old tail with the outer symmetrical, middle point of the new tissue. The new tail stands, therefore, at first at right-angles to the old one, because it is at the beginning a symmetrical structure.

If this analysis is correct how can we account for the subsequent changes that bring about a more rapid development on one side, so

[1]) As shown by Fig. 7 in my paper Regeneration in Planarians. 1900.

that the new tail swings into line with the old one? It is obviously the same phenomenon by means of which the new head of the planarian is carried around into line with the long axis of the old part. The change is due to a more rapid growth on one side, but the factors that bring this about are entirely unknown. The problem is to account for more rapid, or for continuous growth on one side of a symmetrical structure by means of which the new part becomes itself unsymmetrical, but completes an unsymmetrical old part in such a way, that in the end the old and the new supplement each other to form a symmetrical whole. The factors producing these change, while exciting our highest interest, escape, at present, completely our power of analysis; unless we choose to adopt a vitalistic, teleological view of the problem. It is better, I think, to wait, in the hope that the discovery of further facts may make the interpretation possible.

It remains to be shown that the same interpretation can be applied to those cases in which only a part of the tail has been removed by one or by two oblique cuts, Figs. 2 and 14. The new part is here also at first formed symmetrically in the new material, that is, the middle ray of the series exposed at the cut-edge joined by an imaginary line to the symmetrical middle point of the free edge gives the position of the middle ray of the new part that stands, therefore, at right-angles to the cut-surface. The interpretation in this, as in the preceding case, goes no further than to assume that a symmetrical part is first laid down as observations verify. The causal explanation of this phenomenon is unknown to us at present.

Regeneration of the Fins of Fishes.

Weismann in his book on the Germ-plasm states that »fishes are unable to regenerate a lost pectoral or pelvic fin, while the much more highly organised Salamander has been known to regenerate a limb six times in succession«. I examined the power of regeneration in the pectoral and pelvic fins of Ctenolabrus with the following results. The pectoral fin was cut off at about its middle, and it regenerated completely. A pelvic fin had also partially regenerated when the fish was killed. The median post-anal fin also regenerated. The dorsal fin was in part removed, and had begun to regenerate when the experiment was brought to a close. The results show that all the fins in Ctenolabrus have the power of regeneration.

I tried another experiment with the pectoral fins of young individuals of Batracus tau. Regeneration took place very slowly yet a new part was formed. The fish died before I could determine whether or not the fins would have regenerated fully.

The experiments described in the preceding pages show that regeneration of the caudal fin takes place in several genera of teleosts. How generally it occurs in the group must be determined by further experiments.

Summary.

1. If the tail of Fundulus is cut off at any level a new tail regenerates.

2. If the tail is cut off obliquely as shown in Fig. 1 the new tissue forms more rapidly at the part nearer the base of the tail, Fig. 1 *a*. If the tail is cut off by two oblique cuts as shown in Fig. 2 the new tissue grows more rapidly at two points, viz., those that lie nearer the base of the tail. If the tail is cut off by two oblique cuts, as shown in Fig. 3 the new tissue grows faster in the angle. In all cases, therefore, the more rapid growth takes place nearer the base of the tail.

In order to show that the faster growth is not due directly to its nearness of the region to the base of the tail the following experiment was made. The tail of one fish was cut off at its base, Fig. 6 *c*, of another at the middle, Fig. 6 *b*, of a third at the outer end, Fig. 6 *a*, and the rate of growth compared with that of a fish whose tail was cut off, at the same time, obliquely, Fig. 6 *d*. The new tissue in the tail cut across grows equally fast in all three cases. In the tail cut obliquely the part near the base grows at about the same rate as in the tails cut across, but near the outer part the growth is less rapid.

3. If the tail of Stenopus is cut squarely off, Fig. 10 *A*. The new tail, grows faster at two points and produces as a result the typical swallow-tail. If the tail is cut off obliquely, Fig. 10 *B*, the new tissue grows faster at the base where more is needed (teleological view).

4. The tail of Decapterus is more deeply bifid, Fig. 11, and gives similar results, Fig. 11 *A*, *A'*. The tail of Menticirrhus has a ventral lobe, Fig. 12. If the tail is cut squarely off it grows faster on the lower side.

The interpretation of the result seems to be that the new material assumes the typical form before it has reached its full size (causal view).

5. When the tail is cut off obliquely the rays in the new part stand at first at right-angles to the cut-surface. A similar result has been observed in several other animals. The interpretation of this relation is discussed in the text, and the conclusion reached, that it is due to the development of a symmetrical structure in the new part when differentiation first begins.

6. The pectoral and pelvic, dorsal, caudal and anal fins of Fundulus have the power of regeneration.

Bryn Mawr, Penn., U. S. A., Apr. 16 1900.

Zusammenfassung.

1. Wenn der Schwanz von Fundulus in irgend einem Höhenniveau abgetrennt wird, so erzeugt sich ein neuer Schwanz.

2. Schneidet man den Schwanz (nach Fig. 1) schräg ab, so entsteht das neue Gewebe rascher an dem Theil, der der Schwanzbasis näher liegt (Fig. 1 *a*). Wird der Schwanz durch zwei Schrägschnitte, wie in Fig. 2, abgetrennt, so wächst die Neubildung an zwei Punkten rascher wie an denjenigen, welche der Schwanzbasis näher liegen. Liegen die Schrägschnitte wie in Fig. 3, so wächst das neue Gewebe rascher in dem Winkel. In allen Fällen findet daher das Wachsthum am schnellsten an den der Schwanzbasis nächst gelegenen Stellen statt.

Um zu zeigen, dass das schnellere Wachsthum nicht unmittelbar durch die größere Nähe der Schwanzbasis bedingt ist, wurde folgender Versuch angestellt: der Schwanz eines Fisches wurde an seiner Basis abgeschnitten (Fig. 6 *c*), der eines anderen in der Mitte (Fig. 6 *b*), der eines dritten am distalen Ende (Fig. 6 *a*) und das Quantum des Zuwachses verglichen mit dem eines Fisches, dessen Schwanz zu gleicher Zeit schräg abgeschnitten worden war, Fig. 6 *d*. Das neue Gewebe wuchs in den drei quer abgeschnittenen Fällen gleich rasch an. Mit ihnen etwa gleich schnell wuchs der nächst der Schwanzbasis gelegene Theil des schräg abgeschnittenen Schwanzes; näher dem distalen Ende geht das Wachsthum weniger schnell vor sich.

3. Wird der Schwanz von Stenopus quer abgeschnitten (Fig. 10 *A*), so wächst der neue Schwanz an zwei Punkten schneller und bildet so den typischen Schwalbenschwanz. Wird der Schwanz schräg abgeschnitten (Fig. 10 *B*), so wächst das neue Gewebe schneller an der Basis, wo mehr davon benöthigt wird (teleologischer Gesichtspunkt).

4. Der Schwanz von Decapterus ist tiefer zweigespalten (Fig. 11) und giebt ähnliche Resultate (Fig. 11 *A*, *A'*). Der Schwanz von Menticirrhus hat einen ventralen Lappen (Fig. 12). Schneidet man ihn quer ab, so wächst er auf der unteren Seite schneller.

Die (richtige) Ausdeutung des Resultats scheint die zu sein, dass das neue Material bereits die typische Form anzunehmen sucht, bevor es seine endgültige Größe erreicht hat (causaler Gesichtspunkt).

5. Schneidet man den Schwanz schräg ab, so stehen die Flossenstrahlen in dem neuen Theil zuerst unter rechten Winkeln zur Schnittfläche. Ein ähnliches Resultat ist bei mehreren anderen Thieren beobachtet worden. Die Auslegung dieses Verhältnisses ist im Text erörtert und der Schluss gezogen, dass er durch die Entwickelung eines symmetrischen Aufbaues des neuen Theils entsteht, wenn die erste Differenzirung beginnt.

6. Die Brust-, Becken-, Rücken-, Schwanz- und Afterflossen von Fundulus besitzen Regenerationsvermögen.

FURTHER EXPERIMENTS ON THE REGENERATION OF TISSUE COMPOSED OF PARTS OF TWO SPECIES.

T. H. MORGAN.

The experiments that I made a year ago were undertaken in order to find out if regenerated tissue, made up of cells derived from two species, showed any mixing of the specific characters of the two species. For this purpose I grafted the tail of a tadpole of one species of frog upon the posterior end of a tadpole of another species. Later the tail was cut off in such a way (as indicated by the line *b-b* in Fig. 5) that the ectoderm left at the exposed edge belonged in part to one species, in part to the other. When the new tail regenerated there was found to be no mixing of the characters of the ectodermal cells along their line of contact in the new part. The results were unsatisfactory from one point of view, inasmuch as the small piece of ectoderm left after the operation is carried out to the tip of the new tail and increases proportionally less in area than the rest of the new part, so that although it is highly probable that near the tip of the tail new ectodermal cells are being formed by both kinds of ectoderm, still I did not demonstrate that this is actually the case. Moreover, I found that in the later stages the difference in color between the two kinds of ectoderm was less marked than at first, so that the experiment would have been more convincing had the tail been cut off at an earlier stage. This I have done during the present spring, and the results in regard to the ectoderm

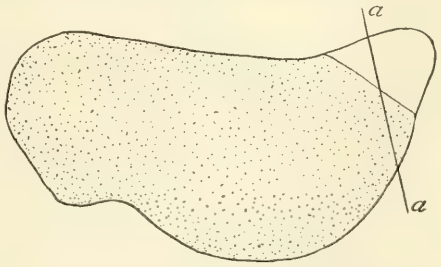
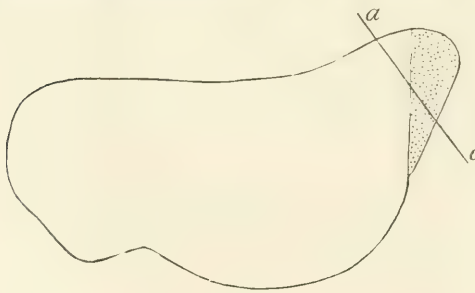


Fig. 1.

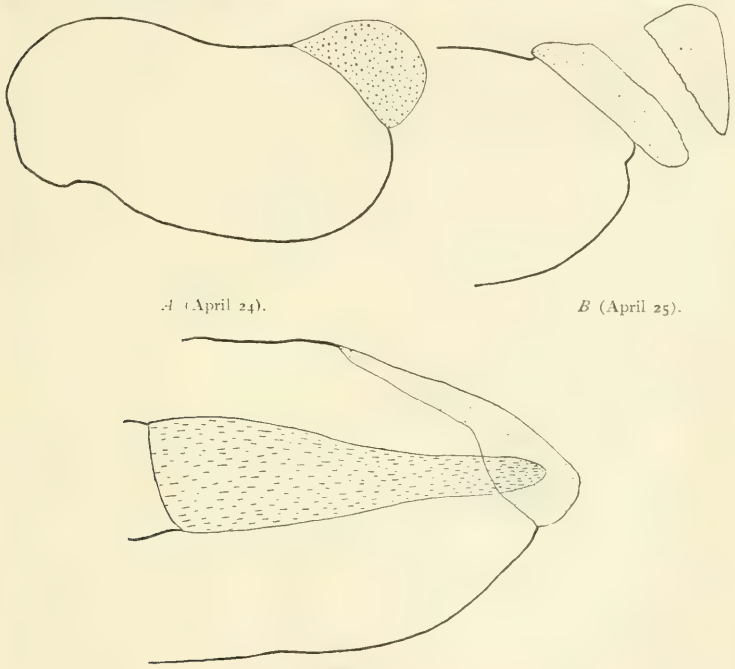
confirm in every way those given in my former paper. In the experiments made this spring my main object has been, however, to carry out the experiment in such a way that there would be left at the exposed edge, when the grafted tail was cut off, the internal tissues of two species. In this way I hoped to be able to determine more definitely if, in the newly regenerated part, the tissues mutually influence each other.

The day after the grafting had been performed (*i.e.*, after eighteen to twenty-four hours) the tail was cut off at the region of union of the two components, as shown by the line *a-a* in Fig. 1. In this way there is left at the exposed edge not only the ectoderm of the two species, but the inner tissues also. The regeneration that takes place from the exposed edge will include material derived from both components. Two possibilities presented themselves. First, would the new part be formed of cells intermediate in character between the two species as the result of an interaction of the cells on each other; or, second, would the new material preserve the characteristics of the region from which it arises, or, in other words, one-half of the tail show the characters of one species and the other half of the other species? It is further possible that the new cells might intermingle, and if so the tail might *appear* to be of a hybrid character.



Other experiments of minor interest have also been studied. For instance, in several cases the grafted tail was cut off after twenty-four hours very near its line of union to the major component, as shown in Fig. 3, *A*, *B*. The experiment was made in order to see if the major component might not have some influence on the regenerated part from which it is separated by only a narrow band of tissue of the minor component, but no such influence was observed.

I have found a much safer criterion than before for distinguishing the inner tissues of the two species of tadpoles used in these experiments. In my former experiments I used the differences in color of the pigment cells. I find that this cannot be relied upon under all circumstances. But the muscle tissue of the tail of Rana palustris is, especially in the early stages, golden-yellow, while in Rana sylvatica the same cells



A (April 24). *B* (April 25).

C (May 2).

FIG. 3.

are slaty-white. The two kinds of cells can be easily distinguished by means of this color difference. Other experiments have shown also that this difference in color is transmitted to the regenerating tissues in the new tail, so that it can be relied upon in the grafting experiments.

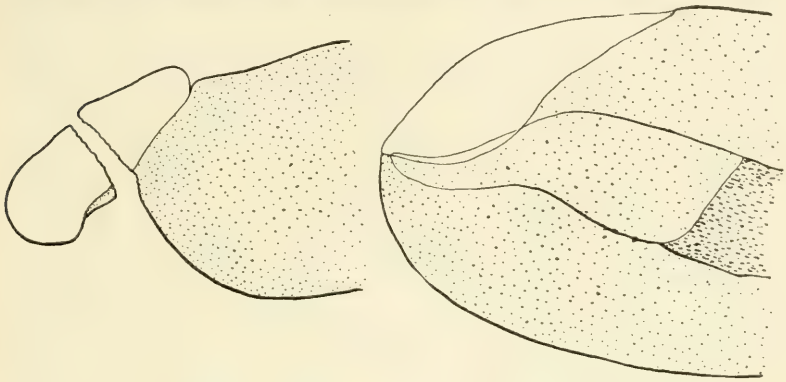
In the first series of experiments the tails were grafted as described in my former paper. After eighteen to twenty-four hours, as a rule, the tail was cut off, as shown by the line *a-a* in Fig. 1. Before grafting it was found more convenient to cut

off the tails obliquely, as shown in the figure — the more anterior end on the dorsal side. Consequently, in order to carry out the second operation of cutting off the tail through the line of union, the cut was made also obliquely, but with the ventral side forward. In a few cases the tails were first cut off with the ventral side further forward (Fig. 2), and the subsequent cutting off was made with the dorsal side forward, as shown by *a-a* in Fig. 2, but the results were practically the same.

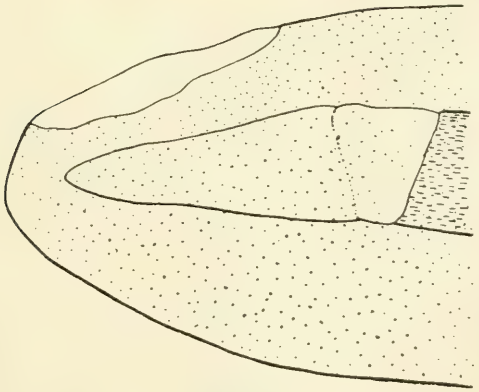
It was found easier to graft the tail of Rana palustris on the posterior end of Rana sylvatica than the reverse. On an average five operations of the former succeeded to one of the latter. The reason for this cannot be given, but it may be due to some difference in the relative sizes of the two components that is more favorable for union in one than in the other way. The result recalls the experiments in cross-fertilization of the eggs in different species, where the crossing can be more easily carried out in one direction than in the other. In this case also the results may be due in some cases to a gross, physical difference, as Pflüger has tried to show for the frog's egg.

In the large majority of cases in which the experiment was carried out as shown in Fig. 1, the core of the new tail seemed to be formed by the minor component, — *i.e.*, if a yellow tail (R. palustris) had been grafted upon a black tadpole (R. sylvatica) and then after twenty-four hours the tail had been cut off obliquely (Fig. 4, *A*), the central part of the new tail would be composed entirely of the yellow tissue derived from the minor component (Fig. 4, *B*, *C*). The small piece of yellow ectoderm is carried out on the new tail and remains near the tip. It covers a larger area than at first, but it increases not nearly so fast as the rest of the new, yellow tissue of the new tail. The distinctive differences in color can only be seen in the core of the tail, *i.e.*, in the cells that form the muscles. On each side of this axial core a broad fin is present containing inside a gelatinous-like substance with scattered cells, but this fin does not show any difference in color in the two species. It is, therefore, probable that in many cases in which the core of the new tail appears to be composed only of tissue from the minor component that the ventral (or dorsal) connective tissue

of the fin is derived from the major component. The differences in the mesodermal pigment cells are at times very striking, and in all such cases the pigment cells are like those in the tissues from which they immediately arise; but while in many



A (April 25). *B* (May 2).



C (May 6).

FIG. 4.

cases they furnish a safe criterion, in others the difference cannot be made out with certainty. On the other hand, the differences in the muscle tissue of the core can always be seen.

The explanation of the result, *viz.*, that the new tail is in most cases (in forty-seven cases out of sixty) like the minor component, is that in nearly all of these operations too large a piece of the grafted tail has been left. It has contained the

notochord and nerve cord and the tissue immediately around them, and from these the new tail has grown out. I did not discover this until most of my material had been used. After this I cut off the grafted tails nearer the line of union, and although regeneration did not take place so well in several cases, still those that regenerated showed more often both parts contributing to the new tail. The same result followed in a number of cases in the previous operations, and we may now examine how regeneration takes place in such cases.

In thirteen cases (out of sixty) there was found evidence of a dual or compound character to the new tail. In all cases observed there was no evidence to show that the duality was the result of the tissues being mixed in character either by commingling of the cells (each cell retaining its specific characters) or by a hybridizing of the cells (due to mutual influence). The duality consisted in each part, regenerating cells like itself, so that definite regions of the new tail were made up of one or of the other kind of tissue. For instance, the new tail might be made up above of the slaty-colored tissue of R. sylvatica and below of the yellow tissue of R. palustris. There is no evidence of a shading of one kind of tissue into the other along the line of meeting, but this point would be very difficult to determine positively. There is further no evidence that the two kinds of tissue are any more commingled at the distal end of the tail than at the base.

In regard to the notochord and nerve cord it is extremely unlikely that the cut would ever pass obliquely through the line of union of the one or of the other, as these structures are very small in cross-section. It is, therefore, probable that in nearly every case the new notochord and the new nerve cord are made up of cells belonging entirely to one component. Furthermore, these two structures lie so near together that it is not probable that the cut would pass between them in such a way that the nerve cord at the exposed edge would belong to one component and the notochord to the other component.

The details of the successful experiments are as follows: On April 14 and 15 nine grafts were made, as shown in Figs. 1 and 2. On April 16 these were cut off, as indicated in Fig. 1, *a-a*,

and Fig. 2, *a-a*, but unfortunately the two lots were not kept separately. On April 29 when again examined new tails had begun to regenerate, and two individuals out of the nine showed that the core of the new tail was compound in character. In both the major component was black and the minor yellow. In one of these the new tail was yellow on the dorsal side and black on the ventral, and in the other the new tail was black on the dorsal side and yellow on the ventral.

In another series the experiment was somewhat different. The grafting took place on April 17. Two days later the ectoderm of the minor component had been carried out further on the tail (Fig. 5), so that at the base of the tail the inner tissues of the minor component were covered by the ectoderm of

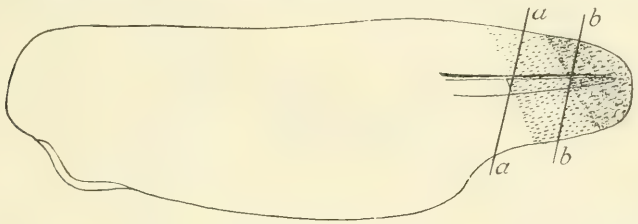


FIG. 5.

the major component. At this time (April 19) the tail was cut off obliquely, as indicated by the line *a-a* in Fig. 4, leaving the inner tissues of both components exposed at the cut surface. On May 19 all three of the tadpoles that had been operated upon showed a compound tail. One of these tadpoles was R. palustris and the other two were R. sylvatica, with grafted tails of the other species, respectively.

In a third series of five individuals, grafted April 19 and cut off April 20, as in Fig. 1, two showed later a compound tail; and in a third I was in doubt whether or not a few of the yellow cells of the major component entered the new tail.

In three later experiments in which the tail had been cut off, so that a smaller piece of the minor component was left attached, a larger number regenerated compound tails.

In one of these experiments the grafting took place on April 27, and the tail was cut off on the following day. One of the three produced a tail composed of both kinds of tissue.

In another experiment grafted April 28 (2.30 P.M.), as shown in Fig. 2, and cut off April 29 (10 A.M.), two individuals formed abnormal tails and a third a compound tail. The tail of this individual is represented in Fig. 6. On the ventral side of the new tail are found the slate-colored cells of the major component, and on the dorsal side the yellow cells of the grafted piece. (It is not possible to show this difference satisfactorily in a simple uncolored drawing, since the principal difference is one of color.) In addition to this difference one can see in the region at which the grafting took place and where the new tissue arises from the old that each component contributes its half to the new tail. Moreover, in all these cases the tadpoles had been carefully observed from day to day (and not only at the intervals recorded in the text) and the gradual formation of the compound tail observed.

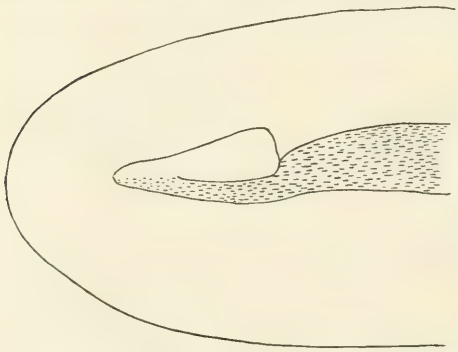


FIG. 6.

In another experiment on April 28 the tail was cut off on April 29. One of the tadpoles did not regenerate a new tail, another (R. sylvatica) had a compound tail, and one had a bifid tail, one branch being compound. Finally in another series in which nine grafts were made, one produced a compound tail, another may have contained a small amount of the major component in the new tail, six regenerated entirely like the minor component, and one was abnormal.

In addition to these cases there were three others (in the total of sixty cases) in which there was an overlapping of the two components in the tail, as in Fig. 6. In two of these the core of the new tail came from the minor component, but it is highly probable that a small addition came from the major component also. In the third case the new tail contained at its more distal end elements from both components. Unfortunately this lot was killed accidentally before they regenerated further.

In several cases double tails grew out enclosed in the same common fin, and lay usually in the same plane. In some cases the core of one of the new tails was derived from one of the components and the other from the other component. In several cases one or the other new tail received material from both components. In one of these cases it could be seen with the greatest clearness that the compound tail received material from both sources (Fig. 7). Cases of this kind are particularly convincing, since they furnish all the data for comparison between the two kinds of regenerating tissue of the two components. The dorsal tail was yellow and the upper part of the ventral tail was also yellow, and its tissue precisely like that of the dorsal tail. The pigment cells also of the yellow component extended out on to both tails.

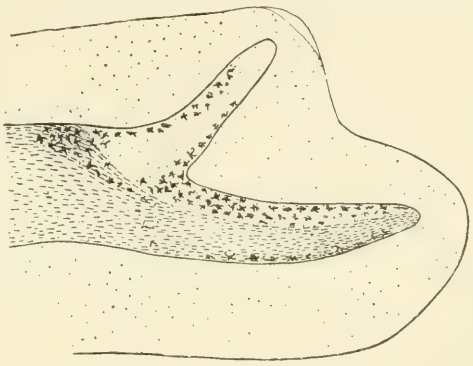


FIG. 7.

These cases of double tails are probably due to imperfect grafting, — the notochord and nerve cord of the two components not being in the same position, so that when the tail is cut off both sets of structures are exposed and a tail develops from each.

Conclusions. — The experiments demonstrate that a single tail may be formed by the regeneration of tissue derived from two species, and that in such cases there is no specific change produced in the one kind of new tissue by the other. Each kind of tissue regenerates its like, and the two kinds combine to form a single morphological organ, — the tail.

May, 1900.

Further Studies on Regeneration in Asterias vulgaris.

By

Helen Dean King.

With 19 text-figures.

Eingegangen am 22. Januar 1900.

The following observations and experiments on regeneration in Asterias vulgaris are a continuation of those recorded in a previous paper (8). They were carried on during the summers of 1898 and 1899 while I was occupying the Bryn Mawr College room in the Marine Biological Laboratory at Woods Holl, Mass.

As a matter of convenience, small star-fish were again used in all experiments. The manner of keeping the animals operated upon and the methods of killing and preserving were essentially the same as described in my former paper. The outline figures given in the text, with the exception of Fig. 4, were made under a LEITZ dissecting microscope with the aid of a camera lucida and then reduced one-half. The regenerating tissue is dotted in all figures to distinguish it from the old.

I.

The following tables give the records of star-fish that were found regenerating one or more arms in their natural environment.

1898.

Date	No. star-fish	No. regenerating
Aug. 4	125	18
- 6	52	4
- 8	20	2
- 9	88	10
- 10	47	5
- 12	26	2
- 13	112	10
- 14	34	5
- 15	103	11
- 16	10	1
- 17	42	5
Total	659	73

% regenerating 11.07.

1899.

Date	No. star-fish	No. regenerating
June 11	77	8
- 13	48	5
- 14	78	9
- 15	99	8
- 16	35	3
- 17	69	8
- 18	182	21
- 19	21	4
Total	609	66

% regenerating 10.83.

During the summer of 1897, 67 or 10.37% of the 646 star-fish examined as they were brought in from the ocean were forming new arms from the disc. Adding this number to that of the later records, it is found that out of a total of 1914 star-fish, 206 or 10.76% were regenerating under natural conditions. New arms were growing out from the disc in every case except one in which a small lateral piece near the tip of an arm had been lost and the missing part was being replaced by new tissue. These observations show that under natural conditions as a rule a star-fish discards an injured arm and regenerates a new one from the disc; only in exceptional cases is a star-fish found in which the distal part of the arm is being regenerated.

Experiments show that the rate of regeneration of an arm is most rapid from the disc and diminishes towards the distal end. This may be due to a greater supply of food in the disc; or, perhaps, to the fact that when an entire arm is removed the injured surface is covered over completely in a few moments by a sliding of the aboral body wall over the exposed surface, producing conditions which are favorable to rapid regeneration. When only a part of the arm is removed, the cut surface is exposed for several days until new tissue is formed at the place of injury, and to this may doubtless be attributed the fact that regeneration is slower than in the previous case. The more rapid growth from the disc would hardly seem to compensate for the loss of an entire arm. Experiments show that an arm from which the tip has been removed either by a transverse or an oblique cut can be of use to the animal in a comparatively short time — a new eye-spot is formed at the cut end in about one week — while the regeneration of an arm from the disc, even one-half the size of a normal arm, requires at least two months.

II.

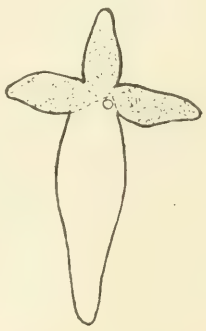
Single arms of Asterias vulgaris cut off close to the disc do not have the power to regenerate the disc, although this power is found in many other species of star-fish according to Haeckel (6), Sars (16), Sarasin (16), and von Martens (9). The arms of Asterias vulgaris show a wonderful vitality, however, as they sometimes live for three weeks, although they can take in no food. To ascertain if the removal of the digestive coeca makes any difference in the length of life of these separate arms, the following experiment was made. Forty arms cut off close to the disc were put into a jar supplied with running sea water, and an equal number of arms from which the coeca had been removed by small forceps were put in another jar also supplied with running water. The two jars were placed side by side and the two sets of arms were kept under exactly similar conditions. No difference whatever could be detected in the behavior of the arms. Those lacking the coeca moved around as actively as did the others, and several individuals in each set lived for eighteen days. The cut edges of these arms drew together after three or four days, but there was no healing over of the injured surface or any regeneration of new tissue. That these arms live some time after being cut off, as does the tail of a tadpole according to Born (3),

shows that the part is »sufficiently independent in its functions to be self sustaining, but whether it regenerates depends on other properties« (MORGAN).

Further experiments were made to ascertain if possible, just how much of the disc must be present in order that a regeneration of the rest of the disc can occur. Out of 175 cases in which single arms had less than one-fifth of the disc attached to them, not one arm lived longer than three weeks. The result was the same whether the part of the disc remaining was directly in front of the arm or at one side of it.

On Aug. 4, 1898, 100 arms were cut off, each having attached to it only as much of the disc as contained the madreporite and the stone canal, this being much less than one-fifth of the entire disc. 25 arms were living at the end of three weeks. On Oct. 6, all were dead but one. In this case (Fig. 1), the single arm was 23 mm. long. The small wedge shaped piece of the disc containing the madreporite had regenerated the rest of the disc with three new arms each 8 mm. long. On examination, it was found that a digestive tract had formed in the disc and that the regenerating arms contained coeca.

Fig. 1.



It is evident from these results that, if a sufficiently large number of experiments are made, it is possible to obtain a regeneration of the disc and new arms from a single arm with less than one-fifth of the disc. That the one experiment succeeded in which the portion of the disc attached to the arm included the madreporite may have been purely accidental; for if a very large number of experiments were made it might be possible to obtain a regeneration of the disc from an arm with a very small piece from any other part of the disc. On the other hand, the region including the madreporite may have a greater power of regeneration than the rest of the disc, and to this, perhaps, may be due the success of the experiment in which the madreporite was retained.

The failure of an arm alone to regenerate the rest of the animal cannot be due to the smallness of the piece, as an arm contains much more material than the disc alone. The latter, if all the arms be removed, will reproduce all the missing members and form a complete individual, as NELSON (12) has observed and as I

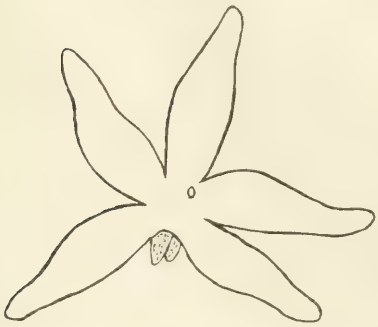
have found true from experiment. One-half of the disc alone does not restore the missing parts, although the experiment has been tried 160 times. The pieces often live for several days but there is never any formation of new tissue. This result is probably due to the great extent of cut surface, rather than to the smallness of the piece.

In experimenting on Planarians, Morgan (11) found that a small piece from any part of the body except the pharynx will regenerate the whole animal. The pharynx alone always dies in the course of several hours. After noting this fact, Morgan adds: »It is probably true that the more specialized a part becomes, the more dependent it is on the rest of the body for certain necessities for its existence, and when removed is unable to exist independently for any length of time.« The failure of single arms of Asterias vulgaris to regenerate the rest of the animal might well be explained on the above hypothesis if we had any positive grounds for asserting that the arms are more highly specialized than the disc.

III.

If a cut be made across the disc from the point of intersection of two arms to the mouth, regeneration is comparatively slow. Previous observations showed that six weeks after such an operation the wounded surfaces have healed over completely, but no new tissue is formed. On Aug. 6, 1898, the operation was repeated on 50 star-fish. At the end of three months 33 animals were still living. In 31 of these the wound had healed entirely, a more darkly pigmented streak across the disc showing where the cut had been made. In the two other cases, however, entirely different results were obtained.

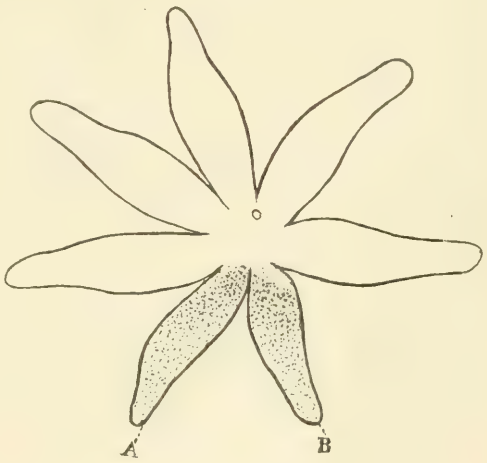
Fig. 2.



In one star-fish (Fig. 2), two new arms were regenerating from the cut in the disc; in the other there was but one new arm. The regenerating arms were apparently normal in every respect, each having an eye-spot, four rows of ambulacral feet and digestive coeca. Star-fish with an extra number of arms are sometimes found in nature. On June 18, 1899, among the 182 star-fish examined, was one

(Fig. 3) with seven fully formed arms symmetrically placed on the disc. Two of the arms (Fig. 3 *A*, *B*) were smaller and more deeply pigmented than the others, showing plainly that they had been produced from an injury to the disc. The stimulus which caused their growth — whatever its nature — was undoubtedly similar to that which produced the extra arms in the experiments above recorded. In Weismann's essay on »The Significance of Sexual Reproduction« he states: »We do not know the causes which have been in operation when a flower possesses one petal more than the usual number, any more than we can explain why it is that one star-fish has five and another six rays. . . .

Fig. 3.



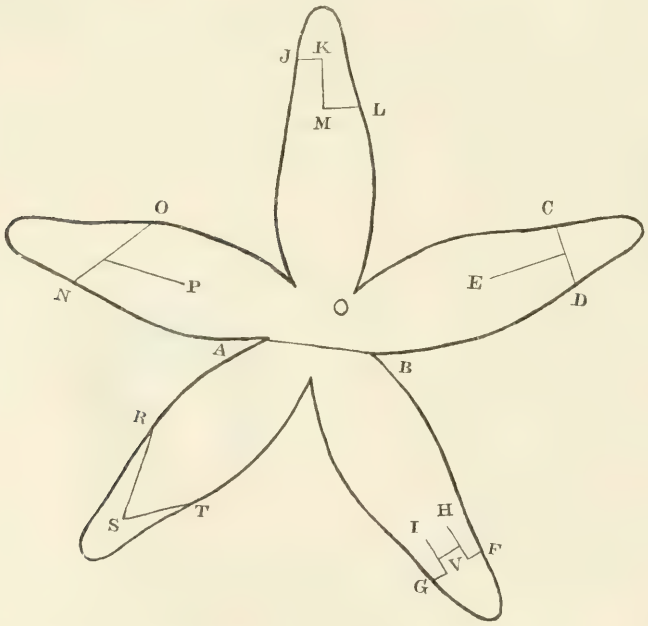
But we can nevertheless maintain in a general way that such irregularities are the result of this complex struggle between the germ-plasms in the ovum and the idioplasms in the subsequent stages of the developing organism, and that they are not the result of external influences.« Egg variations among star-fish by which an abnormal number of rays are produced undoubtedly may occur; but, as far as I am aware, no one has reared such abnormal forms from the egg. It seems to me to be somewhat unwarranted, therefore, to explain the abnormalities found in nature as the result of congenital variations when their previous history is not known. If the star-fish shown in Fig. 3 had been returned to its natural environment the extra arms would undoubtedly have grown to the size of the other arms, and it would be absolutely impossible to determine whether they had been produced by an injury to the disc or whether they were due to egg variation.

Rand (13) has found in Hydra that, »In the case where an excess of oral tentacles was produced during the growth period, the error was promptly righted by a degeneration of two oral tentacles, leaving a normal number«. Such a degeneration of extra members

does not occur in Asterias vulgaris, and even the small pieces of the arms that have purposely been left attached to the larger parts in various experiments (Figs. 10, 12, 13, 14) are not discarded by the animal nor do they degenerate in the course of several months at least.

Weismann might, perhaps, explain the growth of the extra rays from the cut in the disc as due to the fact that »excessive local nutrition has caused the group of determinants in question to become doubled«. If so, what causes excessive local nutrition in

Fig. 4.



one case and not in another? The animals in question were all kept in the same place and therefore under the same external conditions. Rand's view that the production of extra tentacles in Hydra is due to »an accidental effect of the stimulus which must always be present at this period«, hardly seems a satisfactorily explanation of the phenomenon in Asterias vulgaris. Barfurth (2), in experimenting on the regeneration of the limbs of Axolotl, found that in some cases he obtained a regeneration of five fingers on the hand instead of the normal number four. He explains this result thus: »Die verhältnismäßig häufige Regeneration einer fünffingerigen Hand beim

Axolotl ist ein Rückschlag auf die ursprünglich normaler Weise fünffingerige Hand der Amphibien.« Accepting this view we would be justified in concluding from one of the above experiments that the ancestor of Asterias vulgaris possessed seven arms; from a second experiment that six was the ancestral number of arms; while the result of the great majority of experiments would seem to show that this species has retained the original number of arms throughout the whole course of its evolution!

Experiments made by cutting longitudinally through the middle of an arm to the mouth produced no satisfactorily results. The injured arms were thrown off at once or in the course of two or three days and the cut in the disc healed, only one new arm regenerating. In 35 experiments, two arms were removed at the base and then a cut made across the disc to the mouth. The object of these experiments being to ascertain if a more extensive injury would give a stimulus to the production of extra arms in a greater number of cases. The result was disappointing, as in all cases the wound closed and only two arms were regenerated in place of those removed.

IV.

Previous experiments showed that if the madreporite be removed from the disc it will regenerate in its normal position in the course of two months. To ascertain if the madreporite has a fixed position

Fig. 5. Fig. 6. Fig. 7.

in the disc, two arms with less than one-half the disc were cut from 124 star-fish as shown in Fig. 4 *A B*. Four months after the operation all but six of the animals were dead. Three of these were

regenerating but had not yet formed a complete disc with the madreporite. In one case, the madreporite had regenerated in its previous position. In the other two cases, regeneration was taking place as shown in Figs. 5 and 6. In Fig. 5 a complete disc with three new arms has regenerated and the madreporite is on the opposite side of the disc from the position it occupied in the animal operated upon. Regeneration has not been as rapid in Fig. 6 as in the previous case. Only two new arms have regenerated from the disc, and as there is no way of determining where the third arm would have come in, it is impossible to say whether the madreporite has shifted its position or not. Great care was taken in all these experiments to remove the same pair of arms in each case, and the result obtained in Fig. 5 shows conclusively that the position of the madreporite is a variable one and does not depend on some polarity in the disc.

V.

If the tip of a star-fish arm be removed by a transverse cut and the arm split a short distance through the middle as in Fig. 4, *C D E*, two new tips may be formed (Fig. 8) as when a similar cut

Fig. 8.

Fig. 9.

Fig. 10.

Fig. 11.

Fig. 12.

Fig. 13.

is made through the arm without removing the tip; or only one new tip may regenerate in the middle line (Fig. 9). Similar experiments have been made on other animals. VAN DUYNE (5) has found that if the head of a Planarian be cut off and the body then split a short distance in the middle line, two new heads are formed in some cases, while in others the parts grow together or new material is formed

between them and only one new head regenerates. Driesch (4) made a longitudinal cut in a hydroid stem from which the hydranth had been removed. He obtained two new hydranths from the cut ends, each hydranth, however, had a fewer number of tentacles than the individual hydranth removed.

If the distal end of a star-fish arm be removed and a longitudinal cut made through the arm at one side of the middle line, a new tip forms on the larger part which also completes itself laterally (Fig. 10), while the smaller piece, although it remains attached to the arm and heals over, does not regenerate. In 25 cases, after removing the tip of an arm, a longitudinal cut was made on each side of the middle line to see if three new tips would regenerate from the transversely cut surfaces. In all of these experiments, however, the middle part alone regenerated, a new tip being formed in line with the axis of the arm (Fig. 11).

In another set of experiments, the distal end of an arm was cut off transversely in 15 cases and then a section removed as in Fig. 4, *FVG*. Two different results were obtained. In three cases a new tip formed in the middle line (Fig. 12), the lateral pieces healing over but not taking any part in the regeneration. In the remaining cases, regeneration occured as in Fig. 13. The new tip was formed in connection with one of the lateral pieces, the other remaining free. In varying this experiment, sections of the arms were removed as before and then the longitudinal cuts were extended some distance (Fig. 4 *HI*). It was expected that a new tip would grow out from the cut surface at *V*; but in all of the 8 experiments made, regeneration occured as in Fig. 14, a new tip being formed from the middle line in connection with only one of the lateral pieces.

In 39 cases, the tip of an arm was cut off transversely and then a section of the arm removed as in Fig. 4 *JKLM*, to ascertain if two new tips would form from the transversely cut surfaces *JK* and *ML*. In no case, however was such a result obtained. A new tip always formed at the end of the longer piece, and lateral regeneration completely restored the arm to its normal form (Fig. 15).

In former experiments, it was found that if a portion of an arm be removed by an oblique cut, the regenerating tip is at first at right angles to the cut surface and later swings around in line with the rest of the arm. Barfurth (1), Morgan (10) and Hescheler (7) have met with the same phenomenon in experimenting on other

forms. If the tip of a star-fish arm be removed by two oblique cuts, but one new tip regenerates and it is always in line with the rest if the arm (Fig. 16). RANDOLPH (14), in experimenting on Planarians, found that only one new head is formed when the anterior end of the worm is removed by two oblique cuts, although two new heads are formed if a longitudinal cut be made through the anterior part of the animal. In 56 experiments, the distal end of an arm was removed by an oblique cut and the arm then split longitudinally as in Fig. 4 *NOP*, to see if two new tips would regenerate from the obliquely cut surfaces, as is sometimes the result when a tip is cut

Fig. 14.

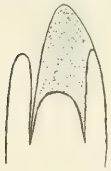


Fig. 15.

Fig. 16.

Fig. 17.

Fig. 18.

Fig. 19.

off by an transverse cut and the arm then split longitudinally in the middle line (Fig. 8). In every case only one new tip regenerated and that always in line with the rest of the arm, whether the longitudinal cut was made through the middle of the arm or at one side. When two longitudinal cuts were made the result was the same (Fig. 17). Oblique cuts made as in Fig. 4 *RST*, also failed to produce two new tips, regeneration in every case taking place as shown in Fig. 18. In varying these experiments, a tip was removed by a transverse cut and then a section of the arm removed by making a longitudinal cut through the middle line and an oblique cut near the disc. Only one tip regenerated (Fig. 19) and this was not in line with the rest of the arm but nearly at right angles to the longitudinally cut surface.

In other experiments, wedge-shaped, semi-circular or rectangular

pieces were cut from the sides of the arms at varying distances from the disc and in every case the missing part was completely restored, the arm appearing perfectly normal save for the darker pigmentation of the regenerated tissue. In similar experiments previously recorded, no regeneration of new tissue was found to take place in the course of two months, the wounded surface was healed over and the arm bent towards the injured side. These former experiments had not extended over a sufficiently long period for regeneration to take place, as lateral regeneration is always much slower than distal regeneration. If the animals live four months after the operation the missing parts are completely restored. If sharp cuts be made on each side of an arm extending nearly to the middle line, no part of the arm being removed, the wounds heal completely and the normal shape of the arm is restored; the same result occurs if a portion if the upper or under surface of an arm be removed. In 7 experiments, a long narrow piece was cut from the dorsal surface of an arm and the missing portion was regenerated in the course of three months (Fig. 7).

In but very few of these experiments were the injured arms discarded by the animal. In fact, mutilation must be very extensive or some injurious substance injected into an arm before these small star-fish will voluntarily throw off an injured member at its junction with the disc. Weismann (18) states in his »Germ-plasm« that: »The restoration of a part which possesses the power of regeneration in a high degree, can only take place as a result of definite injuries which are definitely provided for and not from any kind of injury.« It hardly seems possible that mutilations of the kind above recorded ever take place in nature, yet regeneration occurs in these cases just as readily and completely as in any other. In would seem, therefore, that in Asterias vulgaris at least, the capacity for regeneration is a general property of the organism and not a phenomenon of adaptation.

In conclusion, I wish to express my thanks to Professor T. H. Morgan who has directed my investigations and kindly assisted me in my work.

Summary.

1. Out of a total of 1914 star-fish examined, 206 or 10.76 % were regenerating under natural conditions. One or more arms were growing out from the disc in all cases excepting one in which the distal portion of an arm was being restored.

2. Single arms of Asterias vulgaris live from two to three weeks whether the digestive coeca be removed or not. They are not able to regenerate the rest of the animal.

3. If a piece of the disc containing only the madreporite and the stone canal remains attached to an arm, regeneration of the missing parts occurs in exceptional cases.

4. The disc alone will regenerate all of the missing arms. One-half of the disc does not regenerate owing, doubtless, to the great extent of cut surface.

5. If a vertical cut be made across the disc from the intersection of two arms to the mouth, one or two extra arms grow out from the wound in the disc in a small per cent of the experiments. In a great majority of cases the cut edges come together and unite completely, no extra arms being formed.

6. The madreporite has no fixed position in the new disc.

7. If the arms of Asterias vulgaris are mutilated by cutting wedge-shaped, semi-circular or rectangular pieces from them, the missing parts are regenerated and the normal shape of the arms is completely restored in the course of three months.

Bryn Mawr College, Bryn Mawr, Pa., December 18, 1899.

Zusammenfassung.

1. Von 1914 untersuchten Seesternen zeigten sich 206 = 10,76% in Regenerationsprocessen stehend und zwar bei natürlichen Lebensbedingungen. Die neuen Arme sprossten stets von der Centralscheibe aus, außer in einem Falle, in welchem ein distales Stück eines Armes sich gerade regenerirte.

2. Isolirte Arme von Asterias vulgaris leben noch zwei oder drei Wochen, gleichgültig, ob die Darmblindsäcke aus ihnen entfernt sind oder nicht. Sie sind aber nicht im Stande, das fehlende Thier wieder zu ergänzen.

3. Wenn ein Theil der Scheibe, welcher die Madreporenplatte und den Steinkanal enthält, am Arm hängen bleibt, kommt in seltenen Fällen ein Ersatz der fehlenden Theile vor.

4. Die Scheibe allein ist im Stande, sämmtliche fehlenden Arme zu ersetzen. Eine Scheibenhälfte zeigt dagegen keine Regeneration, was zweifellos in der großen Ausdehnung der Schnittfläche seinen Grund hat.

5. Wenn ein vertikaler Schnitt durch die Scheibe von der basalen Berührungsstelle zweier Arme bis in die Mundöffnung geführt wird, so sprossen in einem geringen Procentsatz der Versuche ein oder zwei überzählige Arme aus der Scheibenwunde. In der großen Mehrzahl der Fälle kommen die Schnittränder wieder zusammen und vereinigen sich vollständig, ohne dass überzählige Arme entstünden.

6. Die Madreporenplatte hat keinen ganz bestimmten Standort an der neuen Centralscheibe.

7. Wenn man die Arme von Asterias vulgaris dadurch verstümmelt, dass man keilförmige, halbkreisförmige oder rechteckige Stücke herausschneidet, so werden die weggenommenen Theile wieder ersetzt und die normale Gestalt des Armes völlig wiederhergestellt; dies geschieht im Laufe von drei Monaten.

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EXPERIMENTS

IN

Regeneration and in Grafting of Hydrozoa

BY

FLORENCE PEEBLES

ERRATA.

Page 12, line 22, for *gread* read *great.*

Page 17, line 37, insert comma after *began.*

Page 21, line 3, for *often* read *after.*

Page 23, line 38, for *Hydranths* read *hydranths.*

Page 24, line 37, for *have* read *has.*

Page 25, Fig. 41, for *T*, *M*, *M'*, *T'*, and *P*, read *t*, *m*, *m'*, *t'*, and *p.* Line 20, for *P* read *p.*

Page 26, line 12, for *B.* read *b.*

Page 34, line 33, for *4 mm.* read *1 mm.*

Page 37, Fig. 60, for *P.* read *p.*

Page 37, line 6, for *a* read *a;* line 9, omit semi-colon after *end* and insert after (*a*); line 20, for *6.11 mm.* read *.79 mm.;* line 23, for *3-5 mm.* read *.4-.6 mm.*

Page 44, Fig. 77, for lower *c* read *c'.*

1. Introduction.

Material for the experiments described in the following pages was obtained first at Wood's Holl, where the experiments were begun in the summer of 1896. Hydractinia, Podocoryne, Pennaria, Eudendrium, Bougainvillia, Parypha, and Clava, were experimented upon. The work was resumed at Wood's Holl for a few weeks in the following summer.

In September 1896 a supply of Cordylophora was obtained from the Chesapeake Bay, not far from Baltimore. By repeated addition of fresh supplies of salt water the polyps remained in good condition and showed great capacity for regeneration until December.

During the winter of 1896/97 Hydra viridis, and Hydra grisea furnished material for experiments, some of which I (Peebles 31) have already described. These two species of Hydra were found in ponds in the vicinity of Bryn Mawr College.

In the autumn of 1898 I had an opportunity to continue these studies at the Zoological Station in Naples. Large supplies of Eudendrium, Pennaria, Tubularia, and Bougainvillia were readily available. Unfortunately Podocoryne and Hydractinia are rare in the Bay of Naples, and Antennularia is difficult to obtain.

In the summer of 1899, at the University of Halle, a series of experiments in grafting were made on Hydra grisea, and Hydra fusca.

The methods used in the experiments are so simple that no description of them is necessary. Where any new method was employed it is mentioned in connection with the experiment in which it was used.

It affords me great pleasure to have this opportunity to express my gratitude to those who have given me valuable suggestions and assistance in obtaining material, and in carrying on these experiments. I wish especially to make known my appreciation of the courtesies of the Marine laboratories of Wood's Holl, and Naples, extended by Professor Whitman and Professor Dohrn. I wish also to thank Professor Grenacher and Dr. Brandes of the University of Halle for their assistance, and Professor T. H. Morgan, whose guidance and interest in my studies have been of great value.

2. Regeneration in Colonial Hydroids: Hydractinia and Podocoryne.

Hydractinia, commonly found on the shells of snails, inhabited by hermit-crabs, consists of several kinds of individuals, and these individuals show, to a marked extent, the power of regeneration.

The colony, as described by Agassiz (1), consists of four different kinds of polyps. The first kind, the reproductive individuals, are the male and female polyps, they bear short spherical tentacles on a rounded head, in the center of which a small mouth is present. The individuals of the second class are especially adapted for protecting the colony, they are capable of contracting rapidly, they have small heads, but the stalk is much longer than that of the other polyps. The third and fourth classes Agassiz calls the sterile forms with long or short probosces. These are found in great numbers, and are adapted for nourishing the colony, they possess a mouth surrounded by long tapering tentacles.

Bronn (6) does not classify the different individuals exactly as Agassiz does. He distinguishes four classes of individuals, the reproductive, the nutritive, the protective or spiral, having no mouth-opening; and a fourth class, the short cone-shaped bodies rising from the branched network or hydrorhiza.

Allman (2) also refers to four classes, the spiral zooids, destitute of mouth, but having a hollow body, the hydranths or nutritive zooids, the reproductive zooids, and the tentacular individuals, which he says are not always present; he considers them abnormal, or a malformation of the spiral zooid.

Collcutt (7) has studied Hydractinia echinata with special reference to the anatomy. She describes the members of the colony as follows: the nutritive polyps or Gasterozooids consist of an elongated stalk bearing a cone-shaped head with hypostome surrounded by two rows of tentacles numbering from ten to thirty, and increasing in number as the animal grows. The second class includes the reproductive zooids or Blastostyles; these are shorter and more slender than the Gasterozooids, and have a small mouth at the summit of a conical head bearing two or more closely approximated rows of tentacles which are rudimentary and knob-like. The third class is composed of the spiral, protective zooids, or Dactylozooids, which bear ten to sixteen knob-like rudimentary tentacles, crowded with nematocysts. The tentacular polyps comprise the fourth class, they have long slender stalks tapering anteriorly to a point; no mouth opening is present.

The species upon which most of my experiments were made was found at Wood's Holl. It corresponds to the description that Collcutt gives for Hydractinia echinata. The nutritive, reproductive, protective, and tentacular polyps were present, and also the low cone-shaped individuals described for this species by Bronn.

The experiments were made on individuals from each class, but the results from those made on the first three classes were more satisfactory than the others. I am inclined to agree with Allman in considering the tentacular polyps abnormal. When isolated they soon die, and pieces of the stalk show no power of regeneration.

In consideration of the fact that the different kinds of individuals of a colony differ from each other in form, while they are organically united, a comparison of the way in which they regenerate is of interest. If the stalk of a reproductive polyp is deprived of its hydranth will it regenerate a new hydranth of its own kind or will a nutritive or protective hydranth take the place of the original one?

For the experiments the reproductive, nutritive, and protective polyps were cut from the hydrorhiza and isolated in small dishes containing sea water. The first set of experiments was so simple that it is unnecessary to give a detailed description of the behavior of each individual. The head was removed from each polyp and the portion of the stalk posterior to the cut, which in all cases bore a cut surface at each end, was isolated and its subsequent behavior carefully observed at regular intervals. In the course of twenty-four to thirty-six hours each individual, showing any sign of growth,

produced a new head similar to the one previously removed. An exception to this rule was never seen. If a head regenerated it was nvariably identical in kind with the original head. Hundreds of individuals were tested, always with the same result. The long tapering tentacles of the nutritive polyp rendered it easily distinguishable from the protective individual with its short knob-like tentacles. The tentacles of the reproductive polyp are also short and knob-like, but the constriction in the stalk just back of the hydranth is peculiar to the reproductive polyps and they can be recognized by this before the reproductive organs develop.

Having determined that each individual invariably regenerates parts of its own kind, another series of experiments was undertaken in order to discover if the power of regeneration in each polyp is the same at different levels of the stalk. On account of the extreme shortness of the reproductive polyp, and the presence of the reproductive organs, it is difficult to divide the stalk into several pieces, therefore the experiments were confined to the protective and nutritive polyps.

In the first experiment the stalk of the nutritive polyp was divided by transverse cuts into five pieces as shown in Fig. 1. The pieces with the exception of the most anterior piece (*1*) were isolated, and the rate and character of regeneration watched. There was a slight variation in the rate but no definite conclusion could be drawn for in one experiment a new hydranth formed on the fifth piece before any tentacles appeared on the second piece, and in another experiment a new hydranth developed on the third piece before the fifth piece showed any new tentacles. The experiment was repeated eight times and each time the new hydranths without exception resembled the original hydranth in kind.

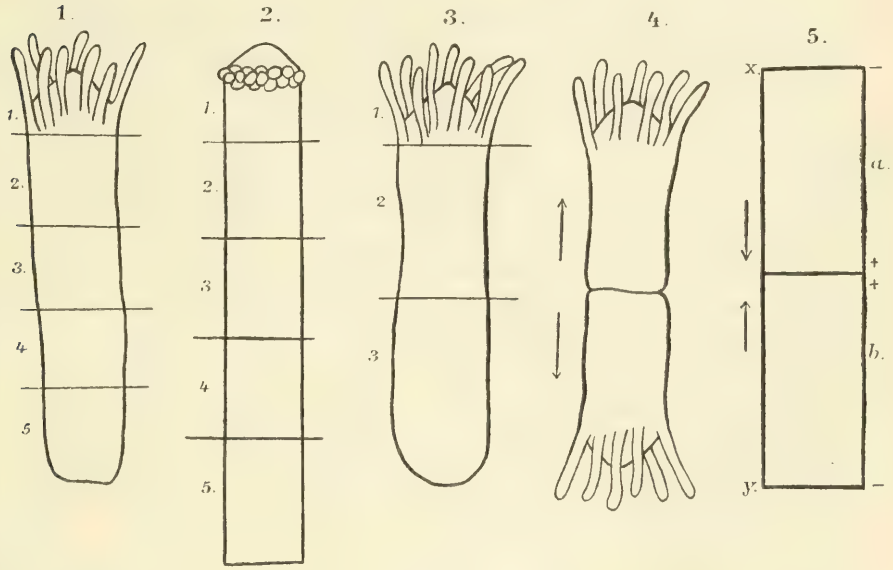
A similar experiment was repeated five times on the protective polyps as seen in Fig. 2. The rate of regeneration in the different pieces varied slightly but with no regularity. The new hydranths were similar in kind to the original hydranth of the polyp from which the pieces were cut.

The experiment was varied by making two transverse cuts through the stalk as indicated in Fig. 3. The first piece was not watched as the object of the experiment was to discover if there was any difference in the rate of regeneration in the anterior (*2*) and posterior (*3*) halves of the stalk. The experiment was repeated twelve times; no regularity in the variation of the rate was observed.

The posterior half (*3*) regenerated new tentacles and hypostome as rapidly as the anterior half (*2*).

In all of the experiments new hydranths frequently developed on both ends of the piece. This was true for all four regions of the stalk, in the protective and also in the nutritive polyps. This heteromorphosis was more frequent in occurrence in small pieces than in those consisting of the greater portion of the stalk [1]). In one experiment fifty-six out of eighty-eight pieces formed hydranths

Figs. 1—5.



on both ends. In another six out of eighteen pieces developed a head at each end.

In many of the experiments on Hydractinia and Podocoryne, especially the latter, when portions of the stalk of a nutritive polyp were left in a dish, undisturbed, and without having any fresh water added, the new growth was limited to sending out stolons instead of developing hydranths. These stolons grew out spreading in all directions; they appeared first at the cut ends of the piece but later growth began from several points along the stalk. The stolons increased in number and in length until a delicate network covered

[1]) This result has been observed in similar experiments made by Bickford (4) on Tubularia and Morgan (28) on Parypha.

the bottom of the dish, and it became impossible to distinguish the original piece of the stalk from the stolons. After several days new individuals began to appear, growing up at right angles to the stolons, just as the different individuals of a colony grow from the hydrorhiza. This formation of new polyps continued for several weeks when the experiments were brought to an end. As many as ten to fifteen new polyps were produced by one piece of the stalk. These polyps were invariably nutritive individuals, characterised by their long slender tentacles.

Pieces from the stalk of the protective polyps were isolated in the same way but the spreading of the stolons was exceedingly slow, and the formation of new individuals was never seen.

In one piece out of many that were cut from the stalks of the reproductive polyps, one new individual developed after the formation of a short stolon. The hydranth on the new polyp seemed to be a nutritive hydranth, but the stolon disintegrated before its character was definitely determined.

Owing to lack of time the colonies, resulting from the experiments described above, were not kept longer than three weeks; it is possible that if they were nourished, and growth continued, the network of stolons would eventually give rise to other kinds of individuals thus forming a complete colony. The spreading of the stolons was much more rapid, and the growth more luxuriant in Podocoryne than in Hydractinia. This was observed in the behavior of those colonies found both at Wood's Holl and at Naples.

Grafting.

In another series of experiments I tried to determine if pieces of individuals, of the same and of different kinds, could be grafted together, and if so whether the subsequent development would be modified. First, I tried to unite individuals of the same class; for this, nutritive polyps were selected as they are larger and more easily handled than the other individuals.

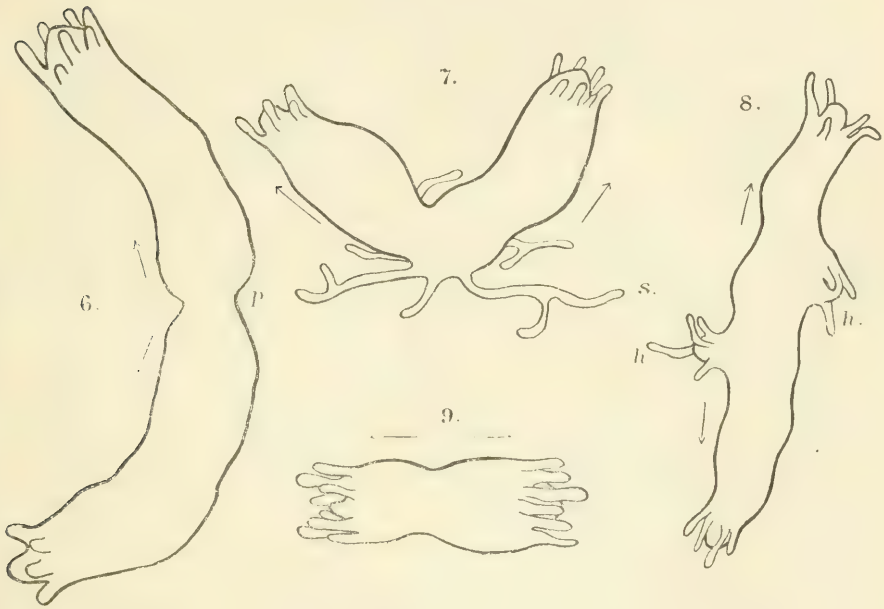
a. Union of nutritive polyps in opposite directions.

For convenience it may be well to speak of the ends of the stalk as poles, the anterior or distal end as the positive (+) or oral pole, the posterior or proximal end as the negative (−) or aboral pole. In all experiments in grafting arrows are used to indicate the direction of the positive pole, therefore they point toward the anterior or hydranth

end of the piece. The union of similar poles may be indicated as shown in Figs. 4 and 5. In Fig. 4, the negative poles are united, in Fig. 5, after the removal of the hydranth the pieces are united at their positive poles.

Exp. 1. Six pairs of nutritive polyps were united at their aboral surfaces as shown in Fig. 4. The polyps had been cut from the hydrorhiza near the base of the stalk. After isolation they are no longer as active as when united with the colony. The shock

Figs. 6—9.

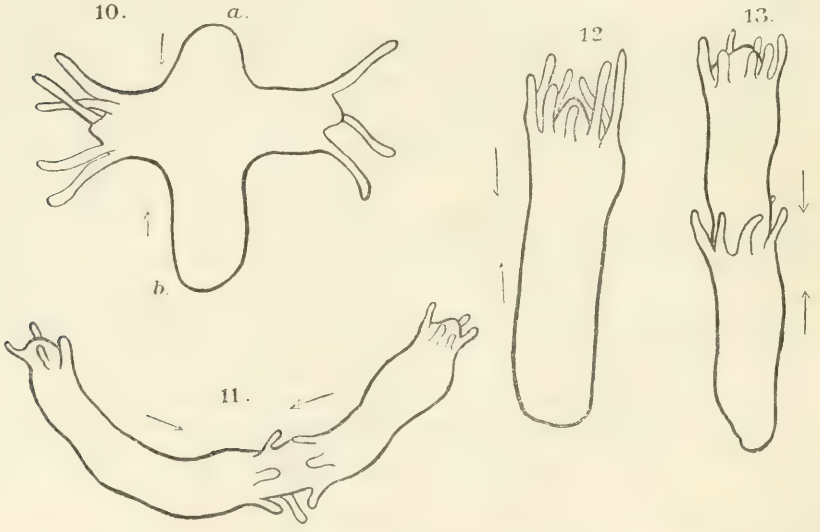


of separation seems to make them inactive so that it is not difficult to keep the pieces in contact until the tissues are firmly united. This complete union results in a few hours, and the line where the pieces are joined soon disappears. Twenty-four hours after the graft was made four of the polyps appeared as seen in Fig. 6, the hydranths forming with each other an obtuse angle, and the common proximal end (*p*) taking a posterior direction. On the following day they were firmly attached at the point (*p*) and at the end of a week had sent out stolons (Fig. 7 *s*) from the region where they were united. The polyps lived for several weeks but no separation of the components took place. The remaining two pairs, instead of forming stolons and becoming fixed at the line of union, developed

two hydranths in this region (Fig. 8 *h*) so that each component bore one hydranth on each end.

Exp. 2. The experiment described above was modified by cutting each hydranth from its stalk just back of the ring of tentacles. The anterior pieces were united as in Exp. 1, but the extent of the common stalk between the two hydranths was much reduced (Fig. 9). After complete union was effected the hydranths remained directly opposite to each other as shown in Fig. 9. In twenty-four hours food was taken and the body cavity was seen to be continuous. The

Figs. 10—13.



stalk increased slightly in length but there was no subsequent attachment in the region where they were united.

Exp. 3. In a third experiment the hydranths were removed by a transverse cut just back of the tentacles. The posterior pieces of two individuals were united as seen in Fig. 5. The negative poles, *x* and *y*, are the regions where the polyps were cut from the hydrorhiza. Sixteen pairs were united in this manner. Several hours after the grafts were made no trace of the line of union of the two components could be detected. At the end of two days new hydranths began to appear but not always in the same place. Six pairs formed new hydranths in the middle, near the line of union (Fig. 10). Three pairs developed mouth and tentacles not only in the middle but also at the aboral ends (Fig. 11). Five pairs formed

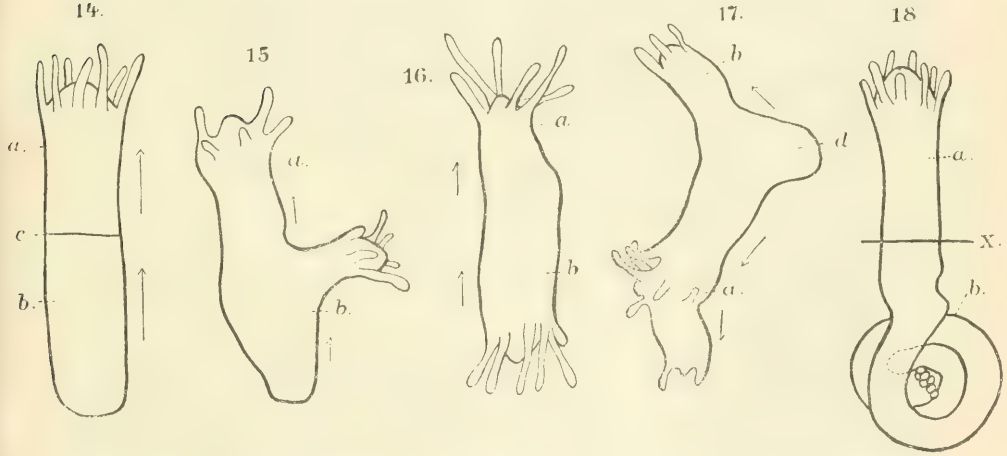
a hydranth at the aboral end of one of the components (Fig. 12). The remaining two pairs produced one hydranth at *x* (Fig. 5), and another at the line of union (Fig. 13).

The great variation in these results can scarcely be due to the region where the cuts were made, for hydranths were produced at both cut surfaces and in regions where no opening was visible, regardless of the position of the components.

b. The union of nutritive polyps in the same direction.

On July 2nd eight pairs of nutritive polyps were united in the manner indicated in Fig. 14. The anterior individual (*a*) was not deprived of its hydranth, but merely cut from the colony near the base of the stalk, and grafted on the anterior end of *b* after the

Figs. 14—18.



removal of the hydranth from the latter. The aboral pole of *a* was thus grafted on the oral pole of *b*. In the course of six to eight hours the union at *c* was complete, the aboral end (*d*) closed in, and one individual of unusual length having a hydranth at the anterior end was formed. On six pairs at the end of two to three days tentacles began to appear near *c* and on July 5th the polyps appeared as shown in Fig. 15. A new hydranth had been produced at the anterior end of *b* but no separation of the two components took place. The two remaining pairs developed as seen in Fig. 16, a new hydranth appearing at *d*, while no new growth was observed in the region where the two individuals were united.

In another experiment two polyps were united in the same way. Three new hydranths were formed in addition to the one already present. Two of these new hydranths developed at the anterior end of *b* and one at the posterior end of *a* (Fig. 17). The component *a* gradually became cut off from *b* but never completely freed itself. After two weeks it was impossible to determine the relation of the new hydranths to the original components.

On July 7th two pairs were united in the manner described in the preceding experiment. On the 8th the union was complete; no new formation was observed. The polyps lived two weeks in the condition shown in Fig. 12; no new hydranths developed, although the cut end (Fig. 14 *d*) healed and the polyps appeared active.

Summary. It is shown, by the experiments described above, that portions of the stalks of nutritive polyps may be grafted upon each other; that the union of the tissues is complete, and that new hydranths may develop at the oral and aboral ends or in the middle near the line of union of the two components. Polyps united as described in these experiments may produce as many as four new hydranths or they may remain alive for one to two weeks without producing new parts.

c. The union of different members of the colony.

Exp. 1. Nutritive and protective polyps.

The complete union of the nutritive and protective polyps was finally effected after many unsuccessful attempts due to the activity of the protective individuals. The experiment was limited to the union of like poles, and the tentacles at first were not removed. A few hours after the polyps were united it was impossible to detect the line of union, but as soon as the nutritive component had taken food its body cavity enlarged while the slender stalk of the protective component retained its normal size. In Fig. 18 the two individuals *a* and *b* are shown. They remained in this condition several days; the nutritive polyp increased slightly in size but no new parts developed. Finally the anterior two-thirds of the nutritive polyp was removed by a cut at the line *x* Fig. 18, leaving a small portion still attached to *b* in order to see if a new hydranth would form at the cut surface, and if so, whether this hydranth would resemble that of the nutritive or protective polyp. As the portion of *a* that was left attached to *b* was exceedingly small I hoped that the new structure would bear some of the characters of the protective polyp. A new hydranth developed at the cut oral surface of *a* but it

resembled the original head. The tentacles were long and slender and the hypostome surrounded a large mouth opening. This experiment was repeated five times with the same result.

Exp. 2. Nutritive and reproductive polyps.

A series of experiments similar to those just described was made with nutritive and repruductive polyps. The reproductive organs were removed before the graft was made, as their presence often causes disintegration of the material. As the nutritive polyps possess apparently the greater capacity for regeneration, and are in every way stronger, than the reproductive, it seemed probable that some influence might be exerted on the reproductive polyp by the nutritive individual. The anterior two-thirds of the reproductive polyp was removed leaving a small portion attached to the base of the nutritive component. A new hydranth formed at the cut surface, resembling that originally borne on the reproductive polyp; the presence of the large nutritive polyp in no way modified the new part. The experiment was repeated four times with the same result.

Summary. The experiments on Hydractinia and Podocoryne show beyond doubt that the individuals of each class retain, throughout the process of regeneration and grafting, the characteristics of the class to which they belong. Although the different kinds of polyps are organically connected, the tissue and structure of each kind is especially modified to perform its individual function, and this modification is never lost.

3. Variations in Regeneration dependent upon the Region from which the Piece is taken.

The experiments made on Hydractinia where the stalk was cut into five pieces (see page 4) seem to prove that in this species the nature and extent of regeneration in each piece is not dependent upon the region from which the piece is taken.

In other forms it has been demonstrated that the character and also the size of the new part is to some extent dependent upon the region where the cut is made. Morgan (27) has found in Allolobophora foetida that short pieces from the anterior end rarely regenerate posteriorly, yet they show great capacity to regenerate in an anterior direction. Very short posterior pieces do not regenerate anteriorly, but pieces from the middle of the worm sometimes regenerate both anteriorly and posteriorly.

I have shown in experiments on Hydra (31) that the rate of regeneration varies according to the region from which the piece is cut. Pieces from the anterior end develop new heads more rapidly than those from the foot region.

In the light of these results it is not possible to accept WEISMANN's hypothesis of latent cells, because it fails to explain the variation in the rate of regeneration in different parts of the body.

In order to determine if the embryonic tissue is qualitatively differentiated in different parts of the stalk, DAVENPORT (9) cut pieces from the branches of Obelia commisuralis at three different levels, and carefully compared the subsequent regeneration. He was lead to conclude that the embryonic tissue was capable at all levels of producing the same thing.

DRIESCH (11) has shown that the hypostome of Tubularia when cut off just back of the distal row of tentacles never regenerated a proximal row of tentacles, while the proximal piece soon replaced the lost hypostome and distal tentacles.

In Hydra I have found that pieces of the hypostome, cut off distal to the tentacles, die without further growth. This result was also obtained when the hypostome of the Scyphistoma of Aurelia was isolated. The pieces showed gread activity but never completed themselves in a proximal direction.

MORGAN (28) has observed that small pieces of Planaria maculata cut from the anterior end beyond the eyes do not regenerate new parts, while pieces including the eyes show a capacity for regenerating the entire worm.

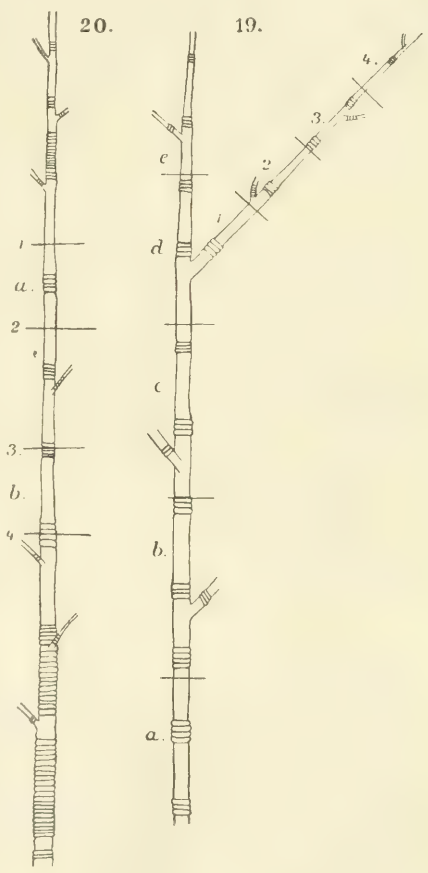
DRIESCH (12) has recently shown that when »not too small« pieces of the stalk of Tubularia are isolated, the extent of the space occupied by the anlage of the new hydranth depends upon the original position of the piece in the stalk. The length of the area occupied by the new hydranth during development decreases from the oral to the aboral end of the stalk.

In order to determine whether the different regions of the stalk of Pennaria showed variation in regeneration I made the following experiments.

In the large species, Pennaria cavolini, found in the Bay of Naples, the stalks measure from twelve to fifteen cm in height and bear strong branches. The longest stalks were selected and cut into short pieces about one cm long. The pieces from each region were

isolated and their behaviour observed each day. Three days after the pieces were cut no change was seen in those taken from the region *a* Fig. 19. In those from *b* six out of seven pieces showed a slight protrusion of the coenosarc beyond the perisarc. The following day five out of seven from the region *c* bore new hydranths, the pieces from *d* and *e* after slight protrusion of the coenosarc died, and those from *a* showed no sign of growth.

Figs. 19—20.



This experiment was repeated twice; in each series twenty-five to forty pieces were observed, and the results from each section (*a*—*e*) showed that the pieces from the center of the stalk regenerated more rapidly than those cut from regions near the base and tip, while pieces from the extreme ends failed to show any growth.

The same experiment was repeated with the branches (Fig. 19 *1*—*4*). Each piece measured from 7—10 mm in length. The pieces from regions *1*, *2* and *3* showed growth and formation of hydranths; those at the extreme tip died soon after isolation.

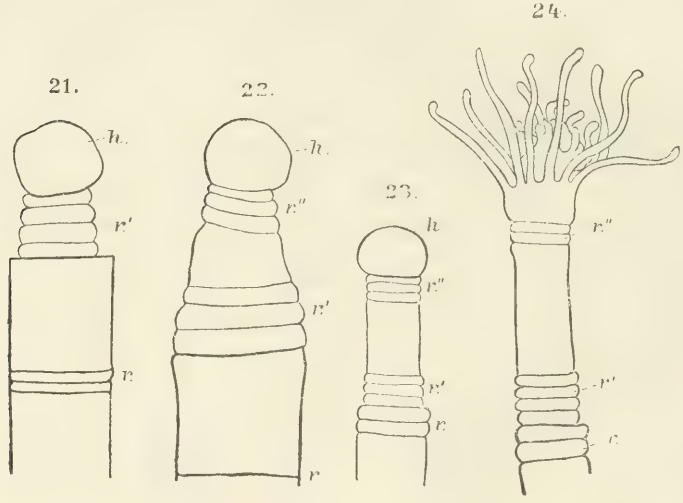
The perisarc of the stalk and branches of Pennaria is annulated at regular intervals. Allman (2) describes the annulation as occuring at the origin of the branches and on the branches themselves; those bearing terminal hydranths are often ringed along their entire length. The species Pennaria cavolini found in the Bay of Naples has groups of three rings just above the origin of the branches.

Agassiz (1) found in Pennaria eight to twelve narrow rings, without any intervals between them, lying at the base of the stalk,

while the region above is only ringed at intervals, and each group is composed of three or four rings. The branches, according to Agassiz, bear three to twelve rings at the base, just beyond the origin of each stalk, and nearer the tip of the branch only three lying below the terminal hydranth.

In the long stalks of Pennaria cavolini, upon which my experiments were made, the annulation of the main stalk was generally limited to groups of three to four constrictions just above the origin of the branches. Stalks were frequently found upon which a region

Figs. 21—24.



of annulation 2—3 cm long was seen. No unsegmented region interrupted these rings. The color of the most anterior segments was slightly lighter than the more posterior segments. Such a stalk is represented in Fig. 20. These accumulations of rings suggested that the stalk might have been broken off at this level, and the new tissue lighter in color had formed above the original rings, thus lengthening the annulated area.

In order to test this view I made a series of cuts along stalks upon which the annulated regions were marked by groups of three or four rings. In Fig. 20 the lines *1—4* represent the position of the cuts. In *a* the piece lying between the lines *1* and *2* was cut so that an unsegmented portion was exposed at each end of the piece, and a group of rings included between the cut surfaces. In *b* the unsegmented region lay in the center of the piece while the

extremities bore two to three rings. The method of regeneration in these pieces was carefully observed in order to determine whether new rings always developed at the exposed surfaces. After recovery from the injury the coenosarc began to grow out beyond the perisarc in the usual manner and the anterior end became round and knob-like prior to the formation of the new hydranth. In a series of three experiments in which thirty-five pieces were cut as indicated by *a* Fig. 20, fifteen pieces developed new hydranths. Fig. 21 shows one of these pieces five days after the operation. At *r* the original rings are seen, at *r'* the new ones forming at the cut surface. As the region between the hydranth and the old perisarc became longer a short unsegmented region appeared between *r'* and the rings immediately below the hydranth (Fig. 22).

In another series of experiments fifty pieces were cut as shown in Fig. 20 *b*. Eighteen of the pieces formed new hydranths. In Fig. 23 one of these pieces is shown four days after the operation. The new rings (*r'*) lie just beyond the cut; the hydranth (*h*) is beginning to develop. On the following day the pieces completed their new hydranths; in Fig. 24 such a piece is shown. Two segmented regions (*r' r''*) have developed in the new tissue, the one at the cut surface (*r'*) has remained at the opening and the new hydranth has a group of rings at its base (*r''*). All of the eighteen pieces that developed new hydranths showed the same regularity in the segmented and unsegmented regions. No great accumulation of rings anterior to the cut surface was observed.

4. The Regulative Influence of External and Internal Conditions on Regeneration.

I. External Conditions.

By modifying the external conditions that surround the organism Loeb (21) has demonstrated a corresponding modification in the rate and character of regeneration.

a) Light. In experiments on Eudendrium Loeb (23) found that changes in the intensity of light produced a marked effect upon the regeneration of hydranths. The greatest number of new hydranths appeared on the branches near the side of the aquarium that was most brightly lighted. Also when cut ends of stalks were placed in great depths of water, where the light was dim, the new formation was slow and imperfect. By shutting out all light the regeneration

of new hydranths was delayed, but when the light was again admitted the growth began at once. In complete darkness LOEB found that the formation of hydranths on the stalks of Eudendrium racemosum rarely took place. In the light, roots grew as luxuriantly as in the dark.

I have attempted through experiments similar to those made by LOEB, to ascertain the effect of light and darkness on regeneration in Tubularia, Pennaria, Bougainvillia, and Podocoryne, and have also repeated LOEB's experiments on Eudendrium.

Small pieces of the stalk were placed in glass dishes containing sea water. All light was excluded by covering the dishes with black boxes. Control experiments were carried on at the same time. In Eudendrium regeneration was delayed, and in ninety per cent of the pieces long stolons formed at the cut ends, while the remaining ten per cent developed hydranths. The majority of the pieces in the control experiment formed hydranths instead of stolons.

Tubularia produced new hydranths as rapidly in the dark as in the light. After the first hydranths formed they dropped off and were replaced by new ones. This process took place slowly in the dark, more rapidly in the light. In the dark, many of the pieces formed long stolons, and in some cases a new hydranth formed at the end of a long stolon-like outgrowth. After the loss of the second hydranths the pieces in the light sometimes produced new hydranths again, but this was never observed in those pieces in the dark.

As the process of hydranth formation in Tubularia is exceedingly rapid, taking place in 24—36 hours, it is not improbable that the darkness has not had sufficient time to modify the formation of the first hydranths, and its influence is exerted only on the subsequent growth.

The production of new hydranths on the cut ends of Pennaria takes place slowly in the light; there was no perceptible change in the rate when the pieces were subjected to darkness, but the percentage of hydranths formed was not so large as in the light.

Bougainvillia shows a marked tendency to produce hydranths at each end of a piece of the stalk. This tendency is as strong in the dark as in the light. In one experiment, in diffuse daylight, sixty-six out of one hundred and seven pieces were biapical. As a control experiment thirty-eight pieces were subjected to total darkness; every piece formed a new hydranth at each end.

The tendency of pieces of Podocoryne to become attached and

to form stolons is so strong that it is difficult to determine whether or not light affects the process of regeneration. Tentacles and hypostome formed as often on pieces in the dark as on those in the light.

Hydra viridis and Hydra grisea, as I have already shown (32), regenerate with equal rapidity in light and in darkness.

Loeb (23) observed that in Eudendrium racemosum blue light has, in a measure, the same effect upon regeneration as darkness. I have placed complete branches and parts of branches of these Hydroids in either red, blue, yellow, or green light. A slight variation in the rate of regeneration was observed, but it was not possible in any one experiment to trace this variation to the character of the light, for as great and even greater variation was seen in the control experiments.

b. Temperature. Lillie and Knowlton (20) have measured the rate and extent of regeneration of Planaria torva, and have proved that cold retards growth while a rise of temperature up to 31.5° C. increases the rate.

The effect of temperature upon regeneration in Hydroids has not been demonstrated, but in Hydra viridis and Hydra grisea I (32) found a rise of temperature up to 32° C. produced a marked increase in the rate of regeneration, and in Hydra viridis under a reduction of temperature to 12° C. the formation of new parts was delayed twenty-four to ninety-six hours.

c. Gravity. According to Loeb, Antennularia reacts to the force of gravity in the following way: Pieces of the stalk suspended vertically in water develop roots on the lower end whether the oral or aboral extremity is directed downward. He found that pieces placed in a horizontal position produce roots on the lower side and branches on the upper side of the stalk.

In order to determine the result produced by constantly changing the relation of the pieces to gravity, I made the following experiment.

Pieces of the stalk of Antennularia, measuring 3—4 cm in length, were fastened in different positions on a large cork. Through this cork a hard-rubber bar was passed, and the bar was revolved at the rate of three revolutions in an hour. The apparatus was placed in a large aquarium through which salt water was constantly running. Five days after rotation of the pieces began new tips measuring 4—5 mm had formed; this tissue grew out in a straight line, but the experiment unfortunately was abruptly brought to an end, so the results were entirely negative.

d. Contact with foreign bodies is an important factor in the determination of the nature of the new part. In Tubularia Loeb (21) found that the regeneration of a new hydranth on either the oral or aboral end of the stem could be prevented by submerging that end in sand. After removal from the sand the hydranth immediately developed.

Driesch (11) sealed the cut ends of pieces of Tubularia to see if in this way by the prevention of new growth at the ends, branches would appear along the sides. The closure of the ends prevented the new formation, and although some of the pieces remained alive, and showed circulation within the perisarc for twelve days, no lateral buds were produced. In one piece a hydranth formed at one end, pushing the wax off and finally emerging from the perisarc.

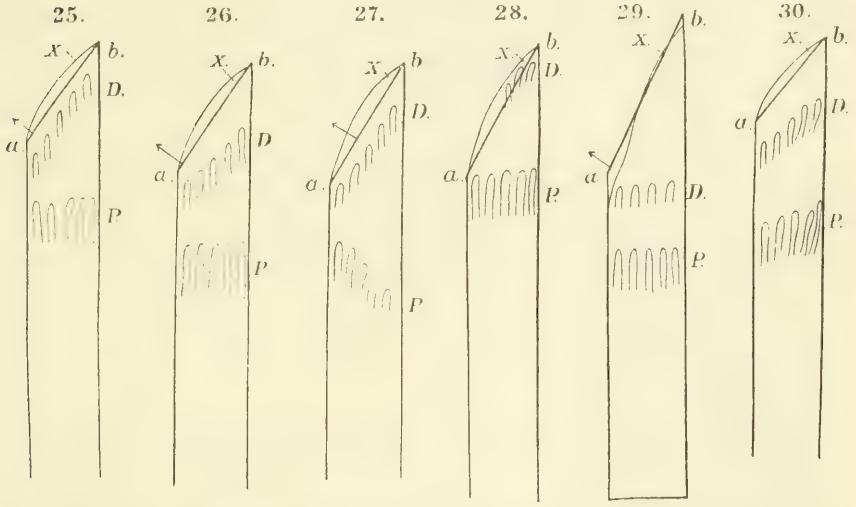
Loeb (22) has demonstrated in Pennaria that when pieces of the stem are placed so that the cut end touches the bottom of the dish in which it is kept, a stolon is produced regardless of whether the cut surface of the piece is a distal or a proximal end. He says that »in Pennaria the nature of the contact not only determines the place of origin of the various organs, but also the direction of growth«. If out-growing hydranths are placed in contact with a solid body, the hydranths grow away from the body, and the new stalk soon becomes nearly perpendicular to the surface with which it comes in contact.

The formation of stolons from small pieces of Hydractinia and Podocoryne, which I have mentioned in a previous section, also demonstrates the effect of contact with a solid body. In one experiment when the pieces were left undisturbed on the bottom of the dish, sixty-six per cent of the pieces formed only stolons; in another experiment twenty-five per cent of the pieces produced stolons. Later all of these stolons sent up new individuals perpendicular to the bottom of the dish. On account of the delicacy of these Hydroids it was impossible to suspend the stalks in the water so that the ends should not touch any fixed surface. Dishes containing the pieces were suspended and swung back and forth, but this change of position was not sufficient to keep the pieces from becoming attached to the bottom of the dish.

A series of experiments on Tubularia have led me to think that the direction of growth of the new hydranth, produced when the stalk is cut obliquely, may be due in part to contact. Driesch (10) cut the ends of stalks of Tubularia obliquely as shown in Fig. 25.

The line *a*—*b* indicates the surface exposed to the water. The position of the ridges which are laid down prior to the formation of the two rows of tentacles, he observed, was altered. The distal row lay nearly parallel to the line *a*—*b*, the proximal appearing somewhat later, and generally parallel to the cut surface, but sometimes it assumed the usual transverse position (see Fig. 25). The new hydranth, according to DRIESCH, grows out of the perisarc at right angles to the cut surface and never comes to lie in the long axis of the piece.

Figs. 25—30.



My own results, obtained from cutting the stalks of Tubularia obliquely, show that the hydranth grows out approximately at right angles to the surface *a*—*b* (Fig. 25) as DRIESCH has already demonstrated, but this direction of growth does not bear, as far as I can determine, any direct relation to the position of the ridges that form the tentacles. The hydranth grows out in the direction of the arrow (Fig. 25) whether the tentacles are laid down parallel to the cut surface or in a transverse position.

The position of the tentacles when they first appear may be due to the shape of the coenosarc in the distal end of the piece, and this seems highly probable in consideration of the fact that the position of the distal row of tentacles is generally more modified than that of the proximal row. In one experiment I cut twenty-four pieces at the angles indicated in Figs. 25—30. In each piece the

coenosarc extended as far as the point x, and in eight pieces the distal row of tentacles appeared almost parallel (Fig. 26 *D*) to the oblique surface; the proximal row (*P* remaining straight. In one piece (Fig. 27) the distal row (*D*) appeared parallel to the cut, while the line of the proximal row sloped obliquely in the opposite direction (*P*). In another (Fig. 28) the distal row formed far out in the acute angle near the tip of the coenosarc in a position very slightly oblique (*D*). The proximal row *P*) lay in a transverse direction. Two pieces cut in an exceedingly oblique direction developed both distal and proximal tentacles parallel to each other (Fig. 29) but not parallel to the cut surface. This may have been due to the fact that only a very small amount of the coenosarc lay in the oblique tip, and therefore the tentacles developed further back. The remaining twelve pieces formed ridges almost parallel to the oblique surface (Fig. 30).

Barfurth (3) observed in tadpoles that regeneration at an oblique surface took place at right angles to the cut, but later the new part came to lie in the same line with the original piece.

Hescheler (16) and Morgan (25) have seen a similar phenomenon in the earthworm, and King (19) has found in the star-fish that after the removal of an arm by an oblique cut the new arm grows out at right angles to the cut, but later comes to lie in the long axis of the original arm.

Morgan (28) cut pieces of Planaria maculata obliquely at each end. A new head and tail formed; these new parts were turned at first obliquely to the median axis of the old part , but later the worm became straight, and head and tail were directly opposed.

I have found that when the heads of Hydra are removed by an oblique cut, the new tentacles form along the line of the cut, but when the new hypostome develops and the new tentacles become longer, the head assumes the normal position in the long axis of the body.

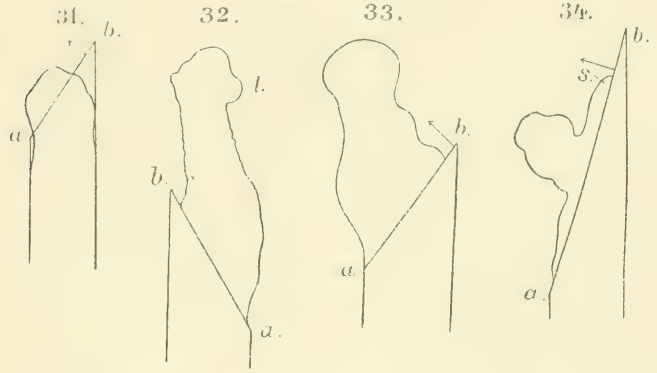
The new parts formed in Planaria and Hydra and other animals such as the earthworm and tadpole, are produced without the limitation of a surrounding perisarc, but Tubularia forms its new hydranth while it is still enclosed in the perisarc. In order to test the effect of cutting the stalks obliquely in species where the hydranth does not form within the perisarc, the following experiments were made.

The stalks of Pennaria and Eudendrium were cut at the angle indicated by the line *a*—*b* Figs. 31—38. In Fig. 31 a stalk of

Pennaria is shown twenty-four hours after the cut was made. The coenosarc (*c*) is not modified in shape as in Tubularia (Figs. 25—30) but is rounded at the anterior end exactly as it is often a cut is made transversely across the stalk.

A later stage may be seen in Fig. 32 where the tentacles are beginning to form (*t*). The new stalk is slightly crooked but the hydranth lies in the line of the original piece. A small per cent of the pieces cut in this way showed a tendency to grow out at right angles to the cut surface (Fig. 33) but the new hydranth with its stalk soon came to lie in the line of the piece.

Figs. 31—34.

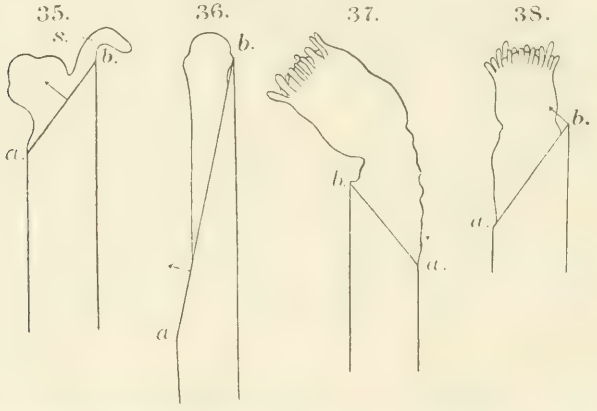


In Figs. 34—38 pieces of the stalk of Eudendrium are shown. Some of these pieces were cut more obliquely than the stems of Pennaria; the difference in the result is probably due to the formation of stolons in front of the hydranths. The line *a*—*b* indicates the angle of the cut, the arrow is perpendicular to the line *a*—*b*, and is used in comparing the variation in the direction of growth of the hydranths.

In four pieces out of fifteen used in one experiment the development of the hydranth took place as shown in Figs. 34 and 35. The direction of growth in the hydranth is almost at right angles to the cut surface, but this seems to be due to the formation of the stolon (*s*) near the point *b*. In three pieces cut so obliquely that the angle at *b* measured not more than 30° the hydranth grew out in the long axis of the stalk as seen in Fig. 36. In the remaining eight pieces the hydranth developed in a line almost directly in the long axis of the stalk (Figs. 37 and 38), but sometimes slightly inclined in the opposite direction from that indicated by the arrow.

In consideration of the fact that in these forms where the hydranth develops outside of the perisarc, there is little connection between the direction of the cut and the position of the resulting hydranth, I am inclined to consider that in Tubularia there is some other factor that influences the direction of growth. The weight of the perisarc in Eudendrium and Pennaria and the branches from each side of the piece enable it to retain the position in which it is placed. In such a piece as that illustrated in Fig. 37 the surface $a-b$ might have been directed toward the bottom of the dish while the same surface in Figs. 34 and 35 lay in the opposite position.

Figs. 35—38.



It may be safely assumed then that the direction of growth in the new hydranth is not controlled by the obliqueness of the cut but is dependent upon some other factor, in this case probably the position of the piece.

e. Slight injuries at any given point may be included among the external conditions influencing regeneration. Loeb (24) cut small slits in the side of Cerianthus, and by preventing the wound from closing, tentacles were produced on the aboral side of the wound.

Having noticed the strong tendency shown by Hydractinia to produce new hydranths at any point along the stalk, I tried to discover if after injuring the stalk in different places by the insertion of a red-hot needle, new growth would take place at the injured region. The wounds made in this manner healed immediately without causing the production of new hydranths. The experiment was repeated with the Scyphistoma of Aurelia with the same negative result.

II. Internal factors regulating regeneration.

a. The size of the piece used in all experiments controls to a certain extent the character of regeneration. I have found in Hydra that under a certain definite size the piece does not regenerate the typical number of organs present in the adult from which it was taken. Pieces averaging $^1/_6$ mm in diameter produced a hypostome and one tentacle, larger pieces measuring $^1/_5$ to $^1/_3$ mm in diameter produced two tentacles, while still larger pieces produced a greater number.

Rand (34) has recently demonstrated that Hydra viridis generally regenerates fewer tentacles than those borne on the adult. This would not account, however, for these small pieces forming only one, and sometimes two tentacles.

b. The shape of the piece. Not only the size but the shape of the piece that is taken from any part of the body modifies the regeneration of the piece. Long narrow strips cut from the wall of Hydra, close in, forming a tube; sometimes they round into balls from which tentacles and hypostome develop. If pieces of the hypostome bearing two or three tentacles are removed, each piece closes in and forms a new hypostome. In this manner small individuals of abnormal shapes are produced. Such pieces, I found, often produce new tentacles around the hypostome, one of the older tentacles in some cases assuming an abnormal position and frequently disappearing. Later, Rand (33) isolated the complete ring of tentacles and found that out of the ring a new Hydra developed, sometimes bearing one of the old tentacles far back on the body. This tentacle usually disappears.

Morgan (28) cut pieces from Planaria maculata in the shape of isosceles triangles, and right-angled triangles, and also long narrow strips. Such pieces are at first abnormal in shape but finally assume the normal form by a rearrangement of old tissue and the addition of new tissue.

The experiments on Tubularia, described on page 30, show that the position of tentacles bears a definite relation to the shape of the coenosarc at the cut end.

c. Modifications produced by interrupting the process of regeneration.

Driesch (11) has found that after interrupting the process of regeneration of Hydranths in Tubularia different methods of repairing

the injury and finally completing the hydranth are followed. After cutting the stalks of Tubularia into pieces 2—3 cm long he waited until the anlage of the two rows of tentacles was visible (Fig. 39 *a* and *b*); then a second cut was made (*x* parallel to the end, separating *a* and *b* so that each piece bore a row of tentacles. The tentacles on *a* developed rapidly into a hypostome, the piece showed great activity, living for several days, but never formed a complete hydranth. The proximal piece (*b*) grew and completed itself distally but this result was reached by different methods. The first method DRIESCH calls »Regenerationsmodus«; here the tentacles pinch off rapidly, the coenosarc pushes out from the perisarc, and the knob-like anterior end forms a new mouth and distal tentacles by a process of budding.

The second method he terms the »Ersatzanlagemodus«. This occurs usually when the cut Fig. 39*x*) is made very near the base of the distal tentacles, leaving a long space between the cut surface and the distal end of the proximal tentacles. A new distal row of tentacles appears in this space and the hydranth develops as if no interruption in the normal process had occurred.

In the third method, the »Auftheilungsmodus«, the proximal ridges divide transversely into two portions, the anterior ends becoming the distal tentacles and the posterior portion the proximal tentacles.

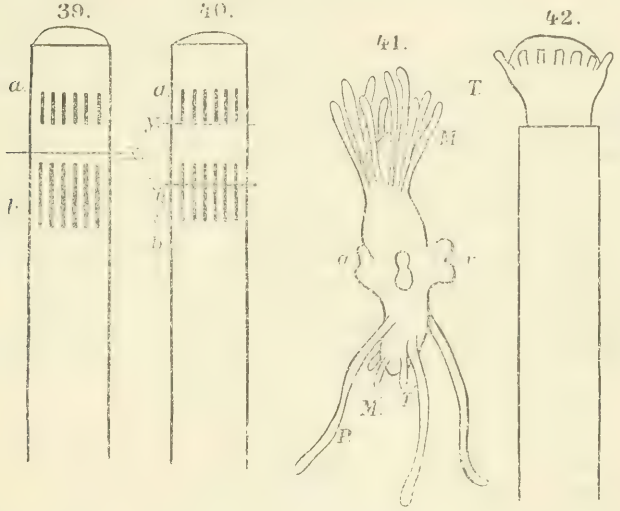
According to the fourth method, the »Auflösungsmodus«, the proximal tentacle ridges disappear by a process of absorption, new distal and proximal ridges are produced and the hydranth develops in the normal manner. The »Regenerationsmodus« was the method most frequently observed by DRIESCH.

In order to determine if the distal piece (*a* Fig. 39) would regenerate in a proximal direction, thus completing a hydranth, I made a transverse cut at *x* Fig. 10, leaving the anterior ends of the proximal tentacle ridges on the anterior piece (*a*). The region where the cut was made was further back on the piece than that in DRIESCH's experiment (Fig. 39*x*). More than a hundred stalks were cut and from these only one showed any development in a proximal direction. In Fig. 11 this piece (*a*) is shown twenty-four hours after the separation from *b* (Fig. 10). The distal row of tentacles (*t* have developed, a mouth (*m*) has formed and the reproductive organs (*r*) have begun to appear. The three tentacles (*p*) back of the reproductive organs are the fully developed distal ends of the original

proximal tentacles (Fig. 40 *p*). At *m'* a new mouth has formed on the proximal end of the piece; this mouth bears three oral tentacles of its own (*t'*); the original proximal tentacles (*p'*) do not become oral tentacles, but remain at the base of the reproductive organs. The tentacles (*t'*) grew longer; no reproductive organs developed between the new mouth and the proximal tentacles (*p'*). The hydranth showed great activity but died without completing the usual number of tentacles.

From this experiment it is evident that when the cut (*x* Fig. 40) is made through the tips of the proximal tentacles just after the

Figs. 39—42.



ridges are definitely formed, it is possible for the distal piece to regenerate new structures in a proximal direction. The fact that Driesch did not obtain this result may be due to the time of development at which the experiment was made or to the position of the cut.

In connection with this experiment it is of interest to determine which end of the proximal ridges (Fig. 40) on *a* becomes the tip and which end the base of the tentacles. As they pinch off from the wall does the end *R* or *S* become the tip? As they usually lie in the position indicated in Fig. 41 *P*, it would seem probable that the original tip (*R*) had become the base and *S* the tip. To determine this several stages in the development should be studied.

The material that I have preserved is unfortunately insufficient for this determination; the tentacles have developed and their position is so irregular that no conclusion can be drawn as to the probable relation of tip and base to the original anlage. The fact that the base of each tentacle in the older pieces lies near the proximal end is in favor of the view that they develop in the usual manner, *S* becoming the base and *R* the tip.

The later development of the proximal piece of the stalk is also modified by the experiment just described (Fig. 40 *b*). If the tentacle ridges are cut apart, as soon as they are visible through the perisarc as indicated in the figure the basal ends of the proximal row on *B* disappear as Driesch has described in the »Auflösungsmodus«, and two new rows form finally producing a new hydranth. If, however, the cut (*x* Fig. 40) was not made until the red ridges were definitely marked off from the surrounding tissue they remained in the position shown in Fig. 42. The coenosarc has pushed out from the perisarc, bearing the basal stumps of the proximal tentacles. The material lying just anterior to the tentacles finally increases in size, and forms a knob-like hypostome upon which new distal tentacles develop. The method of development in this piece corresponds to the »Regenerationsmodus« observed by Driesch. The variations in his results are due, I believe, to the stage of development of the ridges when the cut (*x* Fig. 39) was made, rather than to a different kind of regulative force within the organism.

It is not without interest to note that the basal ends of the proximal tentacles retain their original character instead of becoming oral tentacles for the new hydranth, and that instead of a new row of proximal tentacles developing behind them, a new distal row forms in front (Fig. 42).

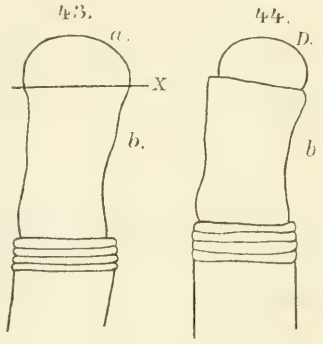
Driesch also cut some pieces close to the base of the proximal tentacles at *y* Fig. 40. I have repeated this experiment on forty-nine pieces; out of these thirty-nine formed a new row of distal tentacles on *b* in front of the proximal row, the remaining pieces lost all trace of the proximal tentacle row but never produced new hydranths.

In such forms as Pennaria and Eudendrium where the formation of the new hydranth takes place outside of the perisarc a similar experiment demonstrates a different method by which an injury is repaired.

Pieces were cut from the stalks of Eudendrium and Pennaria

and isolated until the coenosarc had pushed out beyond the perisarc and the end had rounded slightly as seen in Fig. 43. The distal end was then removed by a cut at x as nearly as possible between the regions where the distal and proximal rows of tentacles would appear. As no trace of the tentacles was visible the cut was made without definite knowledge of its position in relation to the tentacles. After the operation, the distal piece (a) rounded into a ball and died in a few days. The cut end of the proximal piece (b) closed in, and the coenosarc remained apparently passive for several days without further development of the proximal tentacles. On the fourth or fifth day a slight prominence began to appear (D Fig. 44). This knob grew larger and larger until it could no longer be distinguished from the anterior surface of b and the end appeared just as it did before the operation. Development after this proceeded as usual. There was no apparent rearrangement of the old material; all growth and development posterior to the line x seemed to cease until the new tip (D) reached the size of the original tip and the symmetry of the piece was restored.

Figs. 43—44.



5. Grafting.

Trembley (35) succeeded in uniting Hydras of the same species. Marshall (24) repeated these experiments, holding the different individuals together by means of a hair, until the tissues united. Ischikawa (17) united polyps of the same species, holding the components together by means of bristles run through the body. This method was adopted later by Wetzel (37) who has made extended and important experiments on various species of Hydra.

Born (5) has demonstrated the possibility of grafting tadpoles and Joest (18) has made similar experiments on the earthworm. Harrison (14) and Morgan (29) have continued the experiments on the frog larva.

The question whether or not animal tissue of the same or even of different species can be grafted, has been settled affirmatively.

Another problem arising in connection with these experiments is whether the united tissues exert any influence upon each other, and, if so, whether this influence is shown in the subsequent regeneration of either or both of the components, or will it appear in the offspring of the united individuals?

Born's experiments show that in the union of parts of two tadpoles of different species each part retains the characteristics of its own species. Joest also found in the earthworm that even when one of the components was much smaller than the other, the larger piece exerted no apparent influence whatever on the small piece.

Vöchting's (36) results show that in the majority of plants experimented upon no characteristics were transmitted from one part to the other, although modification resulted through the union of annual and biennial varieties.

Harrison (14) interchanged the tails of young tadpoles of different species, and found that in subsequet regeneration the tissues maintained their specific characters.

Morgan (29) has recently made experiments similar to Harrison's in order to discover if the new tissue formed after perfect union of the parts, bears any of the characteristics of the old tissues. In order to determine this the larvae of frogs differing greatly in color were selected. He was able to follow, in this way, the growth of each tissue. After careful observation of the regeneration he found that no modification whatever resulted; each tissue produced new tissue of its own kind.

In the summers of 1896 and 1897, among other experiments I made an attempt to discover if there was any influence exerted by the different individuals of colonial Hydroids when they were grafted together. The experiments have been described in section 3 so that it is unnecessary to give them in detail. They were, I think, conclusive; each individual regenerated tissue of its own kind under all conditions of the experiment.

Later (1898) I tried to bring about some modification in the regeneration of the hydranths of Tubularia by injecting masses of cells from a different species into the body cavity in the following way. After the removal of the hydranth of Tubularia, small quantities of the coenosarc of Pennaria were injected by means of a pipette into the body cavity near the distal end where the new hydranth of Tubularia would develop. Owing to the transparency of the perisarc of Tubularia the mass of Pennaria could easily be seen. Although rapid circulation soon began in the piece of Tubularia the injected

mass remained the same size and apparently none of the cells changed their position. Twenty-four hours after the operation there was no trace of the tentacle ridges and the coenosarc of the Tubularia had grown out beyond the perisarc. The protruded end of the coenosarc was covered with Pennaria cells[1]. On the following day evidence of growth was seen, the coenosarc having extended as much as 1 cm beyond the perisarc, but the mass of Pennaria cells was in the same position. On the fifth day as no hydranth had appeared on either end of the stalk, the anterior end containing the injected mass was cut off. On the following day a new hydranth formed on the aboral end of this piece. The hydranth developed in the usual manner, and was in no way modified by the presence of the cells of Pennaria which remained at the base of the hydranth. The only modification in the result was the first outgrowth of the coenosarc from the anterior end of the piece. This outgrowth was probably due to the effort of the piece of Tubularia to rid itself of the foreign substance. A repetition of this result has proved that the formation of the hydranth on the end of the stalk of Tubularia may be prevented by the injection of cells of another species.

Pieces of the stalks of Tubularia were grafted on the cut ends of branches of Pennaria. Union of the coenosarc lasted for a short time but after the formation of hydranths the pieces disintegrated.

Having failed in the attempt to permanently unite these very different species, a number of experiments in grafting were made with individuals of two species having a close resemblance in form and manner of living. Eudendrium and Pennaria were selected and pieces of the stalks were tied together by means of a cotton thread. The grafted stalks were then placed in a large aquarium where they lived for a much longer period than small pieces live in dishes of salt water. The method of binding the stalks together by a thread along a slender splint of wood was found exceedingly satisfactory. The thread soon wears away in the salt water and drops off taking the splint with it. This does not happen until the tissues have had time to unite. The pieces in this way are supported long enough for them to unite.

When Eudendrium and Pennaria are united together a union of the perisarc does not take place, and the union of the coenosarc is imperfect and only temporary.

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[1] These cells were probably lying around the edge and were carried out by the coenosarc of Tubularia.

When two or more pieces of Pennaria stalks ware grafted together in the same and in different directions, union of the coenosarc results; this union lasts for several weeks, but the perisarc never unites.

HARGITT (13) has recently published the result of experiments made at Wood's Holl on Eudendrium, Pennaria, Parypha, and Campanularia. He succeeded in uniting pieces of the same species after contact of eighteen to thirty-six hours. He held the pieces together and in place by means of small bits of lead until permanent union of the tissues was established. Immediately after the two pieces were placed with their cut ends together, he observed a receding of the coenosarc of each piece within the perisarc, and a closing of the enteric cavity, but later there was a forward extension and final union of the coenosarc of the two pieces, the layer of cells at the ends where the cavities had closed were absorbed, and the two cavities thus rendered continuous.

The perisarc, according to HARGITT, secretes over the line of union a delicate sheet of tissue which may be formed entirely from the perisarc of one end or from both pieces[1]).

He has brought about temporary union between pieces of Eudendrium and Pennaria. The perisarc united but the coenosarc did not. This result is exactly the opposite from that which I obtained from similar experiments on the species found at Naples.

Experiments on Tubularia.

Pieces of equal length united in the same and in opposite directions.

Pieces measuring from 1—2 cm in length were cut from different stalks of Tubularia, and were held together for a short time until the coenosarc of their oral ends was united. Soon after the union was established rapid circulation between the two parts began, and in twenty-four hours the ridges, indicating the position of the new tentacles, were definitely laid down. In nineteen pairs united in this way the following results were obtained.

In five pairs perfectly united two new hydranths formed, one

[1]) I can not discover whether the thickened ring around the region of the graft in HARGITT's figures is intended to represent the perisarc or the lead. I have observed such a thickening of coenosarc but never of perisarc.

on each side of the line of union, and later two more appeared, one on each of the aboral free ends (Fig. 45). As soon as the hydranths at the oral ends were fully developed they pushed out from the perisarc but remained attached by a common hypostome until the pieces died.

Six pairs produced hydranths on each side of the line of union, but the pieces separated before the oral hydranths were fully developed.

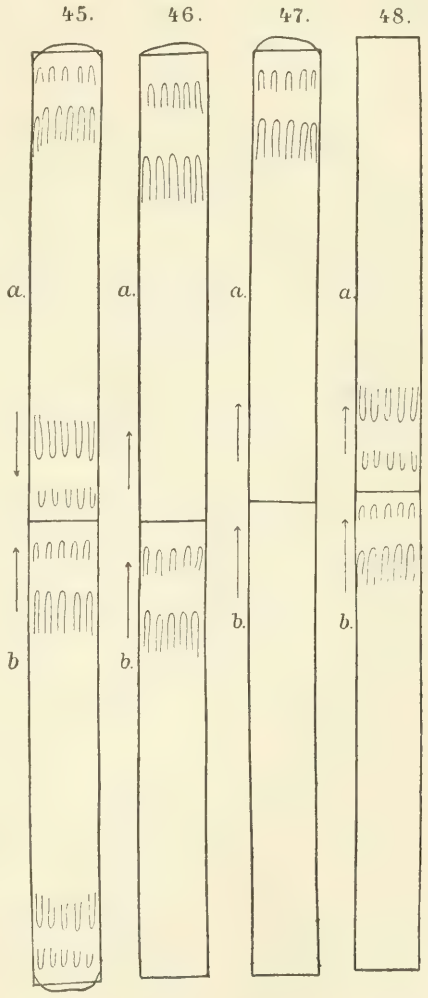
Eight pairs, after perfect union at the oral ends was established, produced aboral hydranths on the free ends first, and later in five cases the oral hydranths appeared.

From these results it seems evident that the union of two pieces at their oral ends does not prevent subsequent regeneration of hydranths at the closed ends. The closing of the end by means of another mass of the same kind of substance does not produce a retarding effect on the formation of new hydranths. The perisarc never unites.

When pieces of equal length were united in the same direction, their unlike poles lying together, the following results were obtained.

In fifteen pairs five rapidly produced new hydranths, one at the oral end of each piece (Fig. 46). As soon as the hydranths were fully developed the pieces separated. Four of the fifteen pairs showed no further growth after they united. In five, a new hydranth was produced on the oral end of *a* (Fig. 47) while *b* showed no trace of the rows of tentacles. In

Figs. 45—48.



this way a single individual was formed by the union of the two pieces. The last pair (see Fig. 48) produced hydranths on each side of the line of union, one on the oral end of *b* and one on the aboral end of *a*. The growth of the coenosarc pushed the hydranths, which were united by a common hypostome, out of the perisarc, and they remained in this condition until the pieces died. It is possible that one of the pieces was reversed, as this result was obtained only once in this experiment.

The union of a long piece with an extremely short one.

Driesch (11) has shown that very small pieces of the stem of Tubularia show great variation in the regeneration of new structures. Out of eighty-two pieces five formed one row of tentacles nearer one end of the piece than the other, twenty-six formed a row at each end; in twenty pieces all the material was used in the formation of a complete hydranth which remained within the perisarc. In twenty-eight pieces one row of tentacles appeared in the middle of the piece, and later the tip of the hydranth and the distal tentacles formed by a process resembling budding. The hydranth finally freed itself from the perisarc.

These variations must be caused by some internal factors regulating the production of the new parts. Recently Driesch (12) has in part accounted for the variation in the behavior of these small pieces. He found that the character of the result frequently depends upon the region of the stalk from which the piece is taken, and also upon the amount of red »hydranth-forming« material present in the piece. This substance increases toward the distal end of the stalk. The smaller the quantity of this red substance, the slower the hydranth is in forming. He says: »Das Schicksal kleinster Stammstücke der Tubularia hängt nicht nur von ihrer Größe, sondern auch von ihrem Ort im ursprünglichen Individuum ab.«

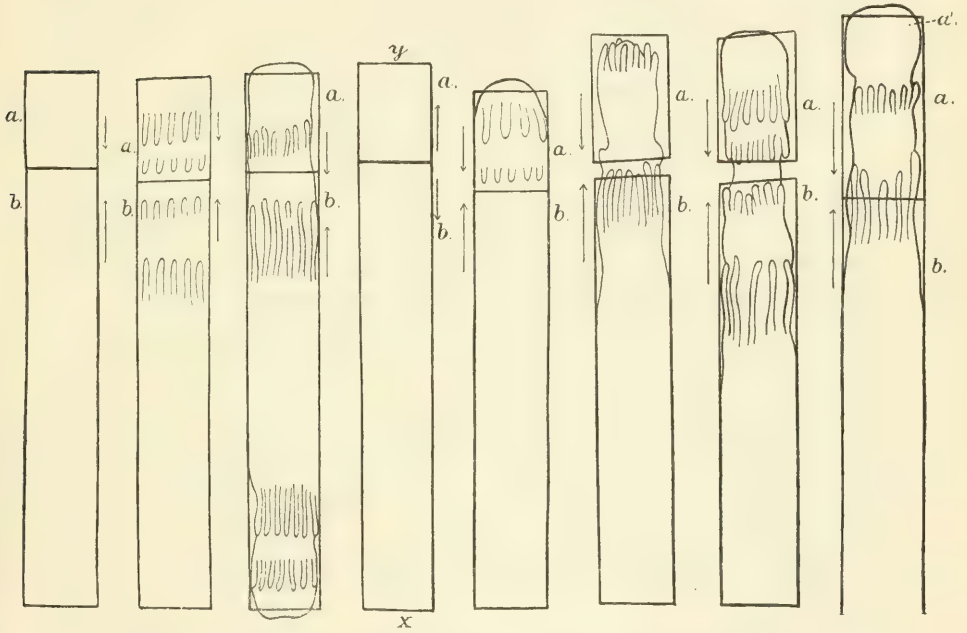
This regional variation led me to carry out the following experiments. A small piece from the region near the base of the stalk was grafted, in a reversed direction, on the oral end of a long piece cut out just below the hydranth. My problem was to determine whether a piece from the basal part of the stalk, when grafted on a distal and more anterior piece, would delay or in any way modify regeneration in the longer piece.

Exp. 1. Grafting a short basal piece on the oral end of a long piece.

From a stalk measuring 3—4 cm in length, the hydranth was removed and a piece (Fig. 49 *b*) about 2.5 cm long was cut from the anterior end.

A small piece (Fig. 49 *a*) of the same stalk, taken from the region near the base, was grafted in the opposite direction on the oral end of the long piece (*b*)[1]. The coenosare of the oral ends united; thus the aboral surface of the two components were exposed.

Figs. 49—56.



On Nov. 3rd five pairs were grafted in this manner; four remained permanently united until the pieces died. The first pair produced two complete hydranths simultaneously, one on the oral end of each piece (see Fig. 50). The subsequent growth of the hydranths pushed aside the perisarc of the short piece and the hydranth *a* freed itself form its perisarc, but remained attached by its hypostome to the hydranth on *b* (see Fig. 57). No hydranth developed on the aboral end of the longer piece (*b*).

The second pair formed hydranths simultaneously at the exposed

[1] The arrows used in all figures point toward the hydranth end.

surface regardless of the reversed direction of the short piece. The hydranth on the end bearing the short piece was not formed entirely from the material in *a* but, as Fig. 51 shows, the distal tentacles formed in *a* and the proximal row in *b*.

The two remaining pairs formed hydranths first on the end to which the short piece was united, the distal row of tentacles lying in the short piece and the proximal in the longer piece. Twelve hours later a hydranth developed on the aboral end of the longer piece (*b*).

The number of times that this experiment was repeated is not sufficient to warrant any conclusion as to the probable influence in the rate of development that either piece exerted on the other. The results indicate a slight delay in the regeneration when the short and long pieces combine to produce one complete hydranth.

An experiment similar to this was recently made on the earthworm. HAZEN (15) has clearly demonstrated that the small piece from the anterior end of the worm, when grafted in a reversed direction on the anterior surface of the posterior segments of the body, produced a head instead of a tail, although the aboral surface of the piece was exposed. The result is not directly comparable with that obtained in the same experiment on Tubularia for in the earthworm the larger piece takes no direct part in the formation of the new head although it nourishes the small piece and thus enables it to accomplish what it could not do alone. In Tubularia, as the figures show, the long piece actually contributes part of its material to the formation of the new hydranth.

Exp. 2. Grafting a short anterior piece on the posterior end of a longer piece.

In order to test the effect of grafting a short piece from the anterior or distal end of the stalk upon the posterior end of a longer piece, the following experiment was made. A piece 1,5 to 2 cm long was cut from the proximal end of the stalk; on the aboral end of this piece was grafted a shorter piece measuring about 4 mm in length, taken from the stalk just below the original hydranth. In Fig. 52 a graft of this description is shown. The aboral ends of *a* and *b* are united, and the oral ends exposed, thus the position of the pieces in the grafts described in the preceding experiment is reversed.

Twelve out of eighteen grafts were successful. Four of the twelve formed a new hydranth at the oral end of the long piece and

none at the oral end of the short piece. Eight formed a hydranth at *y*, one row of tentacles appearing in *a* and one in *b*. Later, after the hydranth at *y* was completed, another developed at *x*, the oral end of the long piece. This seems to indicate that the presence of the small piece from the anterior region of the stalk hastens regeneration at the aboral end of *b*. This may be due to the fact that *a* contributes some of its material to the formation of the new hydranth, although the greater part is developed from the cells lying at the basal end of the stalk. A simultaneous appearance of hydranths at both ends was not observed.

Exp. 3. Grafting a short piece in reversed direction on the oral end of the same piece.

For this experiment pieces of the stalk measuring 2—3 cm in length were cut from the region lying immediately under the hydranth. From the distal end of the piece a short portion was cut and after reversing the position of this short piece it was grafted on the oral end of the same piece from which it was cut.

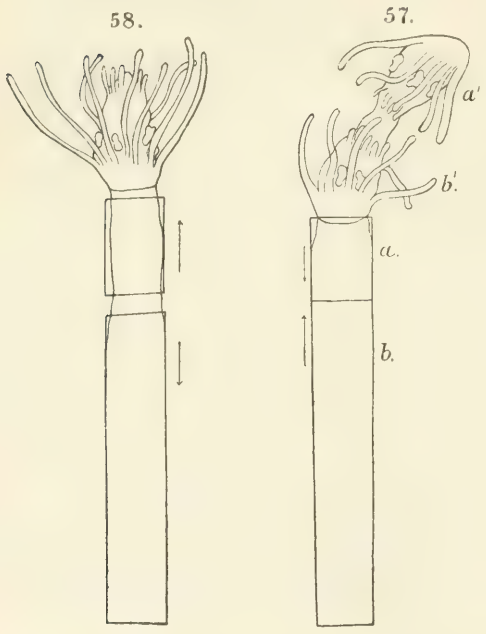
This experiment was performed eighty-eight times; out of this number fifty-one remained permanently united. Thirty-six formed a complete hydranth at one end, the two pieces acted as one, the distal row of tentacles appeared on *a*, the proximal row on *b*, the hydranth was completed, and pushed out from the perisarc passing through the perisarc of *a* as shown in Fig. 58. Careful observations were made at frequent intervals and measurements were taken which prove that the line of union of these pieces lay between the two rows of tentacles. The length of space occupied by the anlage of the hydranth was compared with the length of the perisarc of the short piece. The following table gives these dimensions; the average length of the long piece was 3.5 cm.

Experiment	Length of perisarc a short piece	Length of Regeneration Area
1	.78 mm	1.00 mm
2	1.00 -	.80 -
3	.90 -	1.10 -
4	.80 -	1.03 -
5	.71 -	.90 -
6	.78 -	.90 -
7	.78 -	.60 -
8	.59 -	-
Mean.	.79 mm	.89 mm

In experiments 2. and 7. where the length of the perisarc was greater than the length of coenosarc in which the tentacles developed, the two rows of tentacles lay entirely within the perisarc of the short piece as indicated in Fig. 53.

In six of the fifty-one grafts the tips of the proximal tentacles projected beyond the line of union (Fig. 54) so that they seemed to be formed partly in the short piece and partly in the longer one.

Figs. 57—58.



In five pairs the two pieces developed hydranths independent of each other. The hydranth of the small piece, as seen in Fig. 55, always remained in contact with the hydranth on the oral end of *b*.

The remaining four grafts are shown in Figs. 56, 59, 60 and 61. In Fig. 56 the new hydranth is nearly complete. It has formed from material in both *a* and *b*, but all the material in *a* has not been used as is generally the case when the piece is very short. The coenosarc *a'* remained attached to the hypostome of the hydranth, preventing the mouth from breaking through. The hydranth finally pushed out through the perisarc of *a* but died at the end of five days.

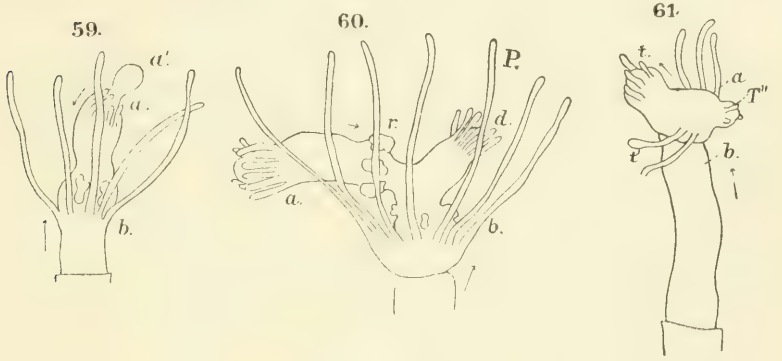
In Fig. 59 another graft of the same kind but somewhat older is shown. The hydranth has emerged from the perisarc bearing the extra material (*a'*) on the tip of the hypostome. The piece *a'* decreased in size from day to day until on the fifth day it reached the size shown in Fig. 59*a'*.

The third graft five days after the union of the two pieces is seen in Fig. 60. The pieces were not evenly united; the result of this is shown in the peculiar relation existing between *a* and *b*. The longer piece (*b*) has produced a complete hydranth with proximal (*p*)

and distal tentacles (*d*). No proximal tentacles are present on *a*. The reproductive organs (*r*) have developed near the base where it is united with *b*.

The fourth and last graft is shown in Fig. 61. The long piece (*b*) has produced a complete hydranth which is concealed by the short piece (*a*). The original distal end of a bears a row of tentacles (*t*) surrounding a hypostome which is united with the hypostome of *b*. Two long tentacles (*t'*) lie in the middle of the short piece (*a*) at the exposed surface of a the original proximal end; a new mouth has developed around which three short tentacles (*T''*) lie. The perisarc originally surrounding *a* has been lost.

Figs. 59—61.



Although great care was taken to reverse the direction of the short pieces it is barely possible that in these cases they were grafted in the same direction as the long pieces. In consideration of the fact that such a result has occurred only several times, it is possible that the pieces were grafted in opposite directions.

The behaviour of isolated pieces of the same length should be compared with that of the short components in the experiments just described. In the table (page 35) the mean length of the short component is 6.11 mm. In nearly all of the pieces used in the experiments given in the table only one row (distal) of tentacles formed in the short piece. Isolated pieces of this length and even shorter (3—5 mm in length) are capable of forming a complete hydranth. This is seen in those grafts where a complete hydranth forms in the short piece.

6. Heteromorphosis.

In the course of experiments made on different species of Hydroids heteromorphosis was observed in Hydractinia, Podocoryne, Cordylophora, Pennaria, and Bougainvillia, and also, as already demonstrated, in Eudendrium and Tubularia.

Experiments on Hydra.

1. Heteromorphosis.

The formation of a new head on the cut aboral surface of the body of Hydra, so far as I can discover, has never been seen. Rand (34) attempted to fasten a Hydra, in an inverted position, to a fixed surface, thinking that a head might form on the free aboral end if the development of a new head on the oral end was prevented. After many unsuccessful attempts he resorted to a method of preventing development at the oral end by grafting pieces of the foot on the open oral surface.

Before describing my experiments in grafting it may be of interest to mention similar attempts which I made (June 1899) to fasten Hydras by the oral end although my object was not so much to prevent development at the oral end, as to hold the polyp so that the cut aboral surface could not come in contact with any fixed object, and develop the characteristic foot.

Large polyps of Hydra grisea or Hydra fusca were selected for the experiment, and were fastened by plunging the oral end in Agar. The Agar was first dissolved in warm water: as soon as it reached a consistency as nearly as possible resembling that of the polyp, the water was poured off and the Agar allowed to cool so that all danger of injury from heat was avoided. The hydra was then lifted by means of fine forceps and plunged head first in to the Agar. Cold water was added, the Agar became hard and the anterior end of the polyp was held firm while the foot protruded from the surface. As soon as the body extended, the foot was cut off thus leaving the aboral surface free.

The experiment was repeated a number of times but was not successful; when the polyps survived the operation they tore themselves from the Agar and soon replaced the lost parts in the usual manner.

2. Heteromorphosis in Grafts.

Having failed in all attempts to produce heads on the aboral surfaces of polyps, a second attempt was made to reach this result through grafting.

Experiments made by WETZEL (34) seem to indicate that it is possible to produce heteromorphosis in Hydra. In one experiment the aboral or foot ends of two polyps (H. grisea) were removed and the individuals held together by means of a bristle until the aboral surfaces united. As soon as perfect union was brought about a transverse cut was made through one of the components just back of the tentacles. Five days later two short hook-like processes formed on the exposed oral surface. These hooks finally formed a foot by which the polyp became attached to a plant. The hydra was killed and sectioned, and the glandular cells of the foot were found at this surface which originally lay just back of the tentacular ring.

A second experiment was carried out by WETZEL in a manner similar to the first, but no cut was made in either of the polyps after their aboral surfaces were united. After the components were firmly united a process began to appear on one side of the body in the region of the line of union. This process soon formed a rounded eminence resembling a bud and later mouth and tentacles developed on its summit. One of the components then began to dwindle into a short hook-like process and the other component with the new bud-like portion remained.

It seems highly probable that this development of a new head at the side of the graft was a bud and that it remained in contact with the body of the mother instead of separating as is usual. RAND (34) has shown by experiment that when nearly mature buds are grafted back into the trunk of the parent, they do not continue to constrict off.

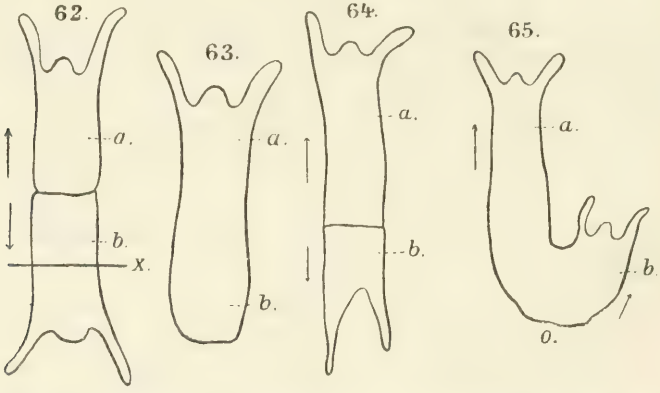
The following experiments on Hydra grisea and Hydra fusca confirm in part, and also extend the experiments made by WETZEL.

Grafting pieces in opposite directions.

In the first experiment the posterior ends of two individuals were removed and the cut surfaces united so that the negative poles were in contact, and the free positive poles bore the original heads (Fig. 62). The results of seven sets of experiments were as follows.

Exp. a. On June 20th two individuals were united as shown in Fig. 62; after complete union a cut was made through *b* at the line *x*. The original head end of *b* was not further observed as the experiment was made for the purpose of discovering the behavior of the cut oral surface of *b*. Three days later the polyp appeared as seen in Fig. 63; the point of union could not be detected, no sign of mouth or new tentacles was visible on the oral surface of *b*, neither was a foot developed instead of a head. Two weeks later, however, the polyp was observed again and was found resting on a leaf but not firmly attached. Sections of the graft were made but no glandular cells were found.

Figs. 62—65.



Rand (34) succeeded in producing a foot on the free oral surface of a very short lateral graft; this he says may be »a possible case of Heteromorphosis«.

Exp. b. On June 21st two pairs were united and cut at *x* in the manner described above (Fig. 62). No tentacles or foot appeared on *b* but in one of the grafts two hook-like processes on the oral surface, such as Wetzel describes, were observed (Fig. 64).

Exp. c. On June 23rd five pairs were united and the anterior end of *b* removed. Four of the five pairs developed a new hypostome and tentacles on *b* (Fig. 65). On the 28th the graft became attached in the region of union (*o*). One pair showed no new structures on *b* and the polyps died without attaching themselves at the closed end.

Exp. d. Two pairs were united on June 28th. No new parts were formed. On July 5th *b* disintegrated and *a* was attached by a new foot on the aboral surface where it was united with *b*.

Exp. e. One of two pairs, united in the same way, showed a similar disintegration of *b* and the formation on *a* of a new foot. The other graft became attached at the oral surface of *b* although no true foot formed.

Exp. f. Two pairs under the same conditions were united on July 7th. On the 13th one pair was attached in the region of the graft as in Exp. e, and b had formed a new mouth and tentacles. The second pair showed no regeneration at the anterior end of *b*. On the 17th the polyps were killed.

Exp. g. On July 13th two polyps were united in the same way. On the 17th a small bud began to form on *a* but no new structures developed on *b*.

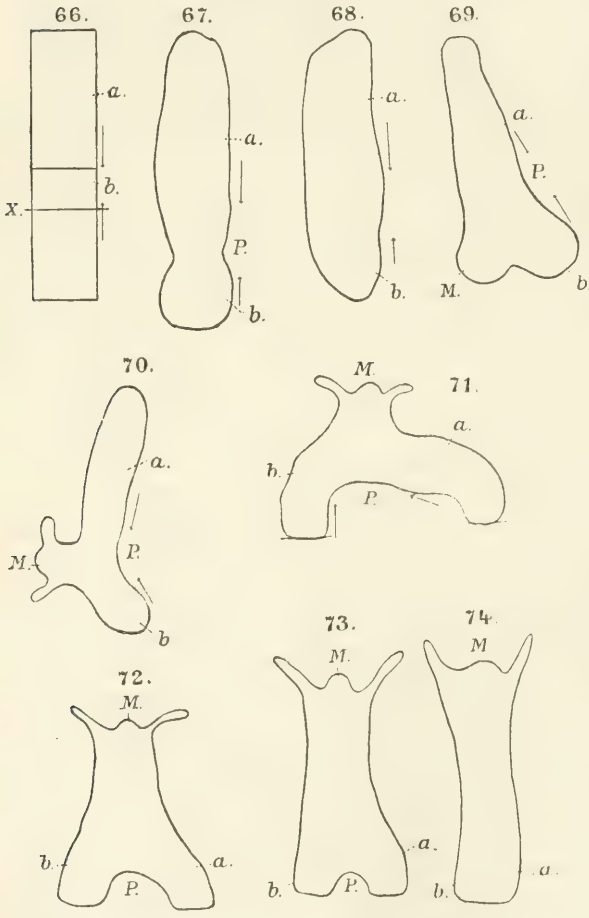
Summary. Fifteen pairs were successfully united at their aboral ends. After the removal of the anterior end of one component, five formed a new head at the cut surface, replacing the lost one and becoming attached at the line of union. Two more seemed to become attached in the same way but showed no regeneration at the cut surface of *b*. Five pairs did not regenerate like or unlike structures after the removal of the anterior half of *b*. Three became attached at the oral surface of *b* but none of the pieces developed the characteristic foot in place of the original head.

2. In the second series of experiments the anterior or oral surfaces of the polyps were united after the removal of the tentacles and hypostome. The direction of the pieces and their relation to each other is indicated by the arrows in Fig. 66. Eight pairs were united in this way, in some the union was perfect, the two individuals forming together a hollow bag-like body, with no visible break in the walls, in others the union was not complete; in places the edges of the two components were open, forming slits. In the eight pairs a new hypostome and tentacles formed invariably at the line of union which represented the anterior regions of the individuals. That the head produced at this point did not belong entirely to one component could be easily seen, for not only a greater number of tentacles were formed than normal for one individual, but in a few cases two mouth-openings appeared. The later fate of such double individuals will be described in the following section.

It has been shown by the experiments just mentioned that although no cut surfaces were exposed, new structures were produced, and there was a decided effort on the part of the double organism to complete itself by the formation of one or two heads in the region

of the union. By another series of experiments I have tried to discover if new heads always form in the region of union if a large surface on one of the components is exposed. The experiments were repeated thirteen times with the same general results. After the complete union of the two components (*a* and *b* Fig. 66) the posterior half of *b* was removed by a transverse cut at *x*; thus the aboral surface of *b* was exposed. A typical result will suffice to describe the behavior of the grafts.

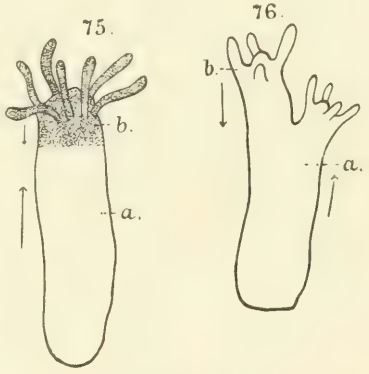
Figs. 66—74.



On June 21st two pairs were united at their oral surfaces as seen in Fig. 66; on the following day the posterior half of *b* was removed. On the 26th the cut surface had healed; a new mouth with its surrounding tentacles appeared near the line of union. On the 27th *a* and *b* were firmly united at their aboral ends while the new head was directed forward. The two bodies were then gradually transformed into one individual having two feet and one head, and finally one head and one foot. This union was brought about in the manner shown in Figs. 67—74. At the point *P* the body of *a* begins to merge into that of *b* by a process which seems to be a forward growth in

the direction of the arrow, and a gradual absorption of the wall between *a* and *b*. In Fig. 67 a slight constriction at *P* renders the region of union distinguishable. The component *b* is less than half the length of *a*; the cut surface of *b* has closed in. In Fig. 68 the constriction has disappeared and the two components form one continuous bag-like body; *a* and *b* can be no longer distinguished unless they differ in color. Fig. 69 shows the new mouth forming on one side (*M*). In Fig. 70 the new head is completed. Up to this time the posterior end of *a* has been firmly attached to the bottom of the dish and the healed aboral surface of *b* has floated freely in the water. In Fig. 71 the new mouth has taken the anterior direction, *b* has bent around until its aboral surface has also become attached and has developed a new foot. The growth or merging of the two individuals into one begins as soon as the head is fully developed; it can be traced by comparing Figs. 71—74. The angle *P* grows smaller and smaller, the feet gradually approach each other, the body back of the ring of tentacles increases in length until, finally, one complete individual is formed; this individual has one mouth and one foot, but generally a much larger number of tentacles than would be normal for one polyp. Twenty-six of the forty grafts merged into one polyp in this way.

Figs. 75—76.



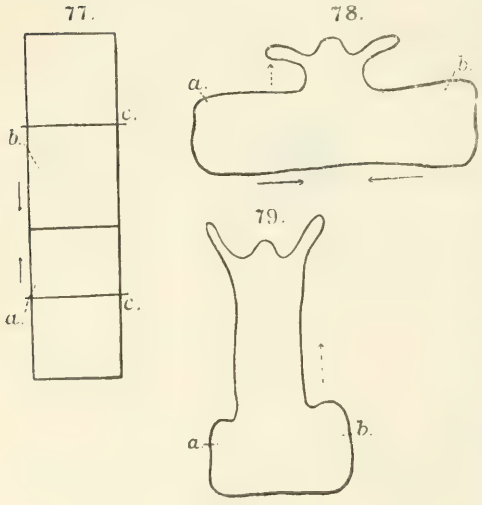
The method described above was also followed when the pieces were united without the subsequent removal of the oral end of *b*. When two distinct heads form at the line of union the two components seem to struggle to free themselves and through this effort many abnormalities result.

On June 19th two individuals were united as described above (Fig. 66); and on the following day a transverse cut was made through *b* close to the line of union of the two components. The cut end closed and for five days no change was visible. On the seventh day mouth and tentacles appeared on the aboral end of *b*, and the new head was composed only of the material in *b* as the line of union was easily seen on account of difference in the color of *a* and *b*.

Fig. 75 shows without doubt that this is a clear case of heteromorphosis, or the formation of a head instead of a foot. This result was obtained only when the cut through *b* was made near the line of union. Five out of twenty-two grafts formed a head instead of a foot. In a sixth graft a head formed on the aboral end of *b* and also on the oral end of *a* as seen in Fig. 76. In the remaining sixteen grafts, if new heads formed at all, they appeared in the region of union.

In another series of experiments where the polyps were united at the oral ends, two transverse cuts were made in order to see if the further development of the new heads would be modified by the presence of two exposed surfaces (see Fig. 77). Thirteen pairs were cut in this way (*c*, *c'*); they developed as follows: The ends healed over but no new structures were developed at either surface; the new head invariably appeared in the middle as shown in Fig. 78. In two grafts this head proceeded to grow forward in the direction indicated by the dotted arrow, at right angles to the original long axis of the body. The forward growth of the new head continued until *a* and *b* became small eminences at the side of the new body (see Fig. 79). The polyps remained in this condition for several weeks but never showed any tendency to become attached by the aboral surface of *a* or *b* or to form a new foot on the lower end of the new polyp.

Figs. 77—79.



In nine of the thirteen grafts a head appeared in the middle at the original line of union; the polyp became attached by the two feet at the posterior ends of *a* and *b*; the two components finally merged into one as described on page 42 where one cut was made instead of two.

Two pairs formed two heads, one on the anterior end of *a* and one on *b*. The two individuals became attached but no separation of the components resulted.

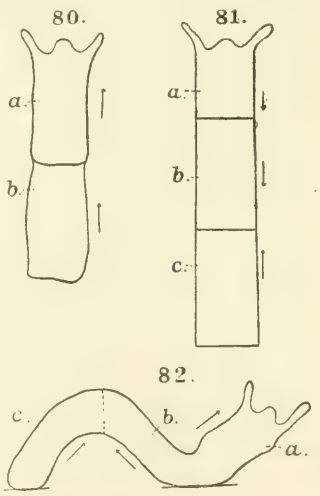
Wetzel (37) has described the merging of two individuals into one. He grafted two polyps together at their oral surfaces after removing the tentacles. The new head appeared near the line of union, the two bodies formed an acute angle with each other, then finally united by a fusion from the apex of the angle.

I have not observed the »wandering« of the smaller component of the graft which Rand (34) describes. The two foot ends seem to be brought together by the forward growth of the new body, not by any migration of the smaller piece.

The formation of one polyp from two pieces united in the same direction.

The experiments just described seem to prove that as a general rule, no matter what the position of the pieces is, or how they are related to one another, the end result is the formation of one complete individual, although the process by which this result is reached may differ with different individuals.

Figs. 80—82.



After the removal of the foot end of one polyp and the head of another the two individuals were grafted so that the aboral surface of *a* was united with the oral surface of *b* (Fig. 80). In a few of the experiments *a* and *b* showed no further growth, the polyp became attached at the base of *b* and, as far as could be seen, the two components formed one individual; however, the body of this individual possessed two budding zones and one reproductive zone (see Fig. 80). In one pair a new head formed at the line of union.

Two single individuals of unusual length were made from the parts of three different polyps; the components were united as seen in Fig. 81. A few days after the experiment the polyp was attached at two points, at the foot end of *c* (Fig. 82) and the combined aboral surfaces of *a* and *b*, the bodies of *b* and *c* forming an arch. In one graft the foot at *x* soon freed itself and the polyps took the normal position indicated in Fig. 81. In the other the component *a* tore apart from *b* and *c*, a new head

formed at the line of union of the oral surfaces of *b* and *c*, and these two components merged into one in the manner described in a previous section.

Summary. The experiments made upon Hydra grisea and Hydra fusca show that no matter in what direction pieces are grafted together they generally form, sooner or later, one complete individual. This result is, in a few instances, brought about by the rapid formation of new structures at the cut surface (Fig. 75) irrespective of the surface exposed. In others the process is exceedingly slow, several weeks elapsing before the two components lose their identity and merge into one individual. These results show a strong tendency on the part of the two components to bring about the normal form but this tendency does not manifest itself in the final separation of the components; on the contrary they seem to strive toward a perfect union by the further fusing of their tissues.

It is of interest in connection with these results to note that when an unusually large number of tentacles develop, in the line of union of the two components, some of them generally disappear as the new polyp is formed. In cases where the new tentacles form a complete ring around the middle of the double body those lying in the angle *P* Fig. 71 are absorbed. Rand (33) has also observed this disappearance of the tentacles when as a result of an experiment they came to lie in abnormal positions.

The later history of these double polyps should be studied. I regret that I could not keep them longer than three or four weeks. It would be of interest to see how and where buds and new reproductive organs appear, for such polyps are composed of a double budding zone, a double foot and no reproductive zone. If no new tissue except that forming the hypostome and tentacles is produced, will the animals remain in an asexual condition sending out buds, but developing no testes and ovaries, or will the new organs regenerate as they do in Tubularia? The process of union between two polyps, forming in the end a single individual, lasts from one to three weeks, ample time for the development of either buds or reproductive organs, but not one of all the polyps showed traces of reproduction by either of these methods.

Summary.

1) Pieces of the stalk of Hydractinia and Podocoryne, when isolated, regenerate a new hydranth on the oral end and a stolon or hydranth on the aboral end; a large per cent of the pieces produce a hydranth on each end.

2) When the stalks of the different individuals of Hydractinia are deprived of their hydranths and are isolated, each individual retains the characteristics of its kind throughout the process of regenerating new hydranths.

3) The rate and frequency of hydranth formation on short pieces of the stalk of Hydractinia is approximately the same at all levels of the stalk. The hydranth forms as soon on the anterior half of the stalk as on the posterior half.

4) Pieces of the stalk of a nutritive polyp of Hydractinia, and especially of Podocoryne, if left undisturbed send out stolons in all directions, and from these stolons new individuals arise, but these individuals always retain the characteristics of the nutritive polyp. Colonies of this sort were kept for several weeks but none of the polyps of the other classes were produced.

5) Two individuals of the same or of different colonies of Hydractinia may be grafted upon each other; the union of the tissues is perfect and lasting. Nutritive polyps may be united with protective or reproductive individuals. In such grafts no influence is exerted by one component upon the other; each retains its own characteristics throughout subsequent regeneration.

6) When two pieces of the stalks of nutritive polyps are united in the same or in opposite directions the subsequent regeneration of new hydranths is rarely ever delayed. New heads appear on aboral and oral surfaces and frequently in the region where the pieces are united. As many as four new hydranths may be produced on one graft.

7) Pieces from the stalk and branches of Pennaria show slight variation in their capacity for regenerating new hydranths. Those cut near the base of the stalk do not regenerate new tissue; the extreme tips show a slight growth of coenosarc but no new hydranths are produced. The most vigorous and complete regeneration takes place in pieces near the middle of the stalk. The pieces from the base and middle of the branches regenerate hydranths, those from the extreme tip show a little new growth but no hydranths develop.

8) The formation of annular constrictions at regular intervals along the perisarc of the stalk takes place in the regeneration of new hydranths. Rings generally appear at the cut surface and later through growth of the coenosarc an unsegmented area forms beyond which another series of rings develops at the base of the new hydranth.

9) Absence of light delays regeneration in Eudendrium and reduces the percentage of new hydranths on Pennaria. Regeneration of new hydranths on Bougainvillia and Tubularia is not affected in the least by darkness. The development of second hydranths on Tubularia may be slightly delayed. Red, blue, yellow and green light do not influence hydranth formation in Pennaria, Tubularia, Podocoryne and Bougainvillia.

10) Contact with foreign bodies produces modification in the regeneration of new tissue. Pieces of the stalk of Hydractinia and Podocoryne form stolons instead of hydranths if left undisturbed in contact with some hard substance.

11) When pieces of the stalk of Tubularia are cut obliquely the new hydranth grows out at right angles to the cut surface regardless of the position of the tentacle anlage.

12) When the heads of Hydra are removed by an oblique cut the new tentacles form along the line of the cut but as soon as the new head is completed it grows into line with the posterior part of the body.

13) If pieces of the stalk of Pennaria and Eudendrium are cut obliquely the new hydranth forms in the normal position; if the growth at first is at right angles to the cut surface the hydranth soon comes to lie in the long axis of the piece.

14) If the anlage of the proximal tentacles of Tubularia is removed from the distal row by a cut just back of the distal end of the proximal tentacles, the anterior piece may regenerate in a proximal direction, forming a proximal mouth surrounded by tentacles.

15) The character of the further development of the proximal piece of Tubularia after separation from the distal piece by a cut between the developing tentacle rows, is dependent upon the stage of development at the time the cut is made. If the tentacle rows are separated as soon as they are laid down the proximal row usually disappears; if the cut is made after the rows are well defined the new hydranth is completed by DRIESCH's »Regenerationsmodus«.

16) If the tip of the hydranth of Eudendrium or Pennaria is

removed before the tentacles form, the tip dies and the proximal piece regenerates a new tip before the new proximal tentacles develop.

17) When a mass of Pennaria cells was injected into the body cavity of Tubularia, the new hydranth forming on the end of the stalk of Tubularia was in no way modified by the presence of the Pennaria. The injected mass usually prevented hydranth formation.

18) Pieces of Pennaria when grafted on Eudendrium show temporary union of the coenosarc but no union of the perisarc. When pieces of Pennaria are grafted upon each other the coenosarc unites and the pieces remain together for several weeks. No union of the perisarc takes place.

19) Pieces of the stalk of Tubularia may be permanently united. The coenosarc of the two components becomes continuous but the perisarc never unites. After union of two pieces of equal length hydranths appear on each side on the line of union or on the exposed surfaces. Pieces united in the same and in opposite directions generally retain their identity, forming separate hydranths.

20) When short pieces are grafted in a reversed direction on longer pieces of the stalk of Tubularia, the short piece frequently takes part in the formation of a new hydranth, the hydranth taking the direction of the long piece, the distal tentacles developing in the short component and the proximal tentacles in the longer component.

21) The experiments seem to prove that when short pieces from the anterior end of the stalk of Tubularia are grafted on the aboral end of a posterior piece the development of the new hydranth on the aboral surface of the longer piece is hastened.

22) Heteromorphosis occurs in Hydractinia, Podocoryne, Cordylophora, Pennaria and Bougainvillia.

23) When two bodies of Hydra grisea or Hydra fusca are united at their aboral surfaces, after the removal of the foot ends, and a second cut is made through one of the components leaving an oral surface exposed, this oral surface may become attached. No characteristic foot forms. A new head usually appears at the cut surface.

24) If two individuals of Hydra grisea or Hydra fusca are united at their oral ends after removal of the heads, a new head develops at the line of union and the two components merge into one. If after the union of two individuals a cut is made through one component, close to the line of union, a new head may form on the aboral surface of the small piece.

25) A single Hydra will form from two pieces grafted in the same or in opposite directions. This process may take place rapidly by the production, at the cut surface, of a head instead of a foot, or a foot instead of a head; or it may take place slowly by a gradual merging of one component into the other.

Bryn Mawr, Pa., May 11th 1900.

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AN EXPERIMENTAL DEMONSTRATION OF THE REGENERATION OF THE PHARYNX OF ALLOLOBOPHORA FROM ENDODERM.

JOHANNA KROEBER.

Several recent investigators have shown with more or less probability that the lining of the new pharynx which develops during the regeneration of the head in certain earthworms comes from the endoderm, while the pharynx of the embryo is lined by ectoderm.[1] It seemed that by means of experimental methods this relation might be definitely determined. In the following pages I shall describe some experiments on Allolobophora foetida that demonstrate, I think, that the lining of the new pharynx is in fact derived from the endoderm.

Hescheler showed, as the result of observations made principally on Allolobophora terrestris, that when the five anterior segments are cut off the pharynx is regenerated by a growing forward of the old digestive tract up to the third segment, and that the new buccal cavity occupying the first three segments is formed by an ectodermal invagination. The old pharynx was not completely removed in these operations, since in the normal worm its cavity frequently extends beyond the fifth segment and its thickened muscular dorsal wall always goes back into the sixth, so that Hescheler's results are open to the objection that in his experiments *a part, at least, of the old pharyngeal wall always remained behind* as a possible source for the regeneration of the new pharynx.

Rievel in experimenting on certain Lumbricidae (Allolobophora foetida, Allolobophora terrestris, Lumbricus rubellus) cut off anterior ends consisting of between one-third to two-thirds of the entire body. He arrives at the conclusion that the pharynx is regenerated from the walls of the digestive tract

[1] See Hoffman, *Zeit. f. wiss. Zool.* Bd. lxvi. 1899.

at the point where this was cut, and that no ectodermal invagination whatever occurs, the endodermal diverticulum joining the body wall to form the mouth at the very anterior end of the worm.[1]

Haase showed that in Tubifex, when four to six anterior segments have been removed, the pharynx grows forward out of the walls of the digestive tract and meets an ectodermal invagination of somewhat varying size. This ectodermal pouch, which forms the buccal cavity, is small in all cases, never extending quite as far back even as the region of the cerebral ganglion.

Von Wagner's observations on Lumbriculus show that the point of union of ectoderm and endoderm, originally at the anterior end of the animal, subsequently comes to lie more posteriorly, on account of the forward growth of the "Kopflappen" and accompanying turning in of the ectoderm.

The differences in the accounts cited above show clearly that it is almost impossible to determine with certainty, merely by observation, just how much of the regenerated pharynx ultimately arises from the ectoderm and how much from the endoderm. It is very easy to see where ectoderm and endoderm meet, but the point of fusion is lost soon afterwards, and since the regenerated head continues to increase in size, it is presumably possible that the point of union may come to lie at some distance from its original position. At the time when the pharynx opens to the exterior its walls are not sufficiently developed for one to be able to determine whether the muscles will grow around the endodermal part of the tube ; but if in some manner the fusion of the ectoderm with the endoderm could be delayed long enough for the pharyngeal muscles to form around the latter, then the origin of the pharynx might be determined. Hescheler affirms that it is possible to make out the exact limits of the ectoderm by using stains which bring out the cuticle covering this layer. I used the stain which Hescheler mentions as giving the best results, but found that, while my preparations showed in general an agreement

[1] For criticism of Rievel's results, see papers by Morgan (*Roux's Archiv*, Bd. v, 1897) and Hescheler (*Jenaische Zeitschrift*, 1898).

with the figures of Hescheler in regard to the extent of the cuticle on the dorsal wall of the pharynx, they contained also some alternating patches of what seemed to be cuticle and of ciliated areas on the ventral wall and at points in the digestive tract even further back than the regenerated pharynx itself.

For this reason I have attempted to get more certain results by the use of the following experimental methods. Worms, from which the seven anterior segments had been removed, so that no part whatever of the old pharynx was left behind, were allowed to regenerate for a period of between twelve and eighteen days. As a rule the fusion of the ectodermal invagination with the pharynx occurs about fifteen days after the removal of the anterior end of the worm, — although there is considerable individual variation in regard to this point, and also some difference due probably to the temperature, etc. At the end of this time the anterior tip of the new part of the worm was removed in one of two ways: either it was burned off by touching it with a hot needle, or it was cut off with fine scissors. The latter method, though more difficult to carry out successfully, proved to be the better one because the piece cut off could be preserved to show whether the pharynx had joined the ectoderm at the time of the second operation. The worms were once more allowed to regenerate and were finally killed between ten and fifteen days after the second operation. In all cases the worms survived both operations and showed a perfectly normal regeneration, — the only point of difference from worms that had undergone only the first operation being that the new pharynx had time to regenerate before the second ectodermal invagination had fused with its anterior end. The object of the experiment was to determine whether a normal pharynx would develop from the endoderm if the fusion of the ectoderm with it was prevented for a sufficient length of time to allow this development to take place.

It is difficult to determine on the living object whether or not the ectodermal invagination has met the endoderm, and since for my purposes it was best to wait as long as possible before the second operation, it happened in two or three cases,

as sections of the small pieces removed showed, that ectoderm and endoderm had met. In the majority of instances, however, I was fortunate enough to remove the invaginating ectoderm just in time. In cases where this was done with a hot needle there is, of course, nothing to prove that the fusion had not taken place. There is ground for such a belief, however, in the fact that, of a number of worms whose small anterior ends were cut off at the same time after the first operation as when the burning was done, and which were kept under exactly the same conditions, there was not a single one in which the fusion had taken place.

Fig. 1.

The same difficulty presents itself again in determining the time at which the worm is to be killed. I succeeded, however, in getting a number of cases where, though the pharynx and the ectoderm were just on the point of joining, they had not quite done so when the worm was killed. The accompanying figures show two worms in this condition.

Fig. 1 shows a vertical longitudinal section of a worm from which seven segments were removed on January 15. On February 2, that is to say eighteen days later, the tip of the newly regenerated part was cut off. This piece was preserved and sectioned and was found to include the whole of the ectodermal invagination besides the anterior end of the pharyngeal diverticulum which had not yet broken through to the exterior. Fourteen days after this operation, on February 16, the worm was killed.

Fig. 2 represents a vertical longitudinal section of a worm from which the first seven segments were cut off on January

15. The tip of the regenerated part was destroyed with a hot needle on February 2, and the worm was killed on February 17.

Both figures show the diverticulum which has grown out from the walls of the oesophagus about to open to the exterior by fusion with the ectodermal pit; and a comparison with the sections in the same neighborhood shows that these two represent the nearest approach of ectoderm and endoderm to be found in the two specimens. The walls of the pharynx and its musculature, especially on the dorsal side, are well developed. In both worms a nerve cord and a cerebral ganglion have been formed, the latter for the second time. Owing to the slight obliqueness of the section, as shown in Fig. 2, the nerve cord is cut for only a part of its length. The muscles of the body wall have begun to differentiate and there are clear indications of metamerism. All of the worms used in this set of experiments, as well as all those in a later set made to test these results, present a similar condition of things.

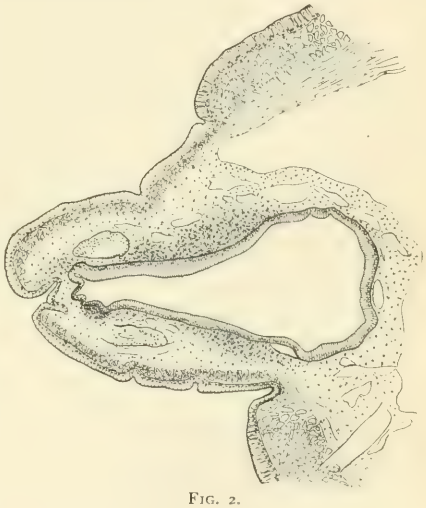

FIG. 2.

From these results we must conclude that the lining of the pharynx is regenerated from the endoderm, while the new ectoderm turns in for a very short distance to meet the pharynx and form the mouth.

The objection may be raised that the possibility of a later pushing in of the ectoderm to form the ultimate lining of the pharynx is in no way removed. But there is no evidence for such an occurrence and, even if it did take place, the fact remains that the musculature of the pharynx develops around an endodermal tube, as my experiments have shown, while in

the embryo the lining of this same region is derived from the ectoderm.

The preceding work was done under the direction of Prof. T. H. Morgan, to whom I wish to express my indebtedness.

BRYN MAWR, May 26, 1900.

A STUDY IN THE HELIOTROPISM OF CYPRIDOPSIS.

By ELIZABETH W. TOWLE.

[*From the Laboratory of Biology in Bryn Mawr College.*]

GRANT[1] in 1829, in the course of some notes on the effect of light on Medusæ, made the following observation: "From the soft gelatinous texture of such beings, it seems natural to expect that an agent impinging on them with such velocity and in so great a quantity as the rays of light, and which penetrates their whole substance, should be able to affect them in some manner, were it only with impressions of touch." "The physical distribution of the lowest species of fixed and nerveless animals is principally determined by the intensity of light." This, with the exception of Trembley's observations on Hydra in 1744, is the earliest suggestion that I have found of the importance of the influence of light on animal forms. But workers in physiology have been very slow to study the problems here suggested; and not until quite recent years has any careful work been done in this direction.

Bert in 1868[2] and Lubbock in 1884[3] made a number of experiments to determine the "color preferences" of different forms; but in this work they took into consideration only wave lengths, color as such, leaving out other important factors, such as the intensity and direction of the light rays. In 1883 and 1884 Graber[4] made an exhaustive series of experiments in which he attempted to solve the problem whether and in how far animals are in a condition to distinguish intensity and color differences. He made a distinct advance on his predecessors in that he considered the influence of varied

[1] GRANT, R. E.: Edinburgh journal of science, 1829, x, p. 347.

[2] BERT, P.: Mémoires de la société des sciences physiques et naturelles, Bordeaux, 1868, vi, pp. 375–383.

[3] LUBBOCK, J.: International scientific series, 1884, xlii, New York.

[4] GRABER, V.: Sitzungsberichte der kaiserl. Akademie der Wissenschaften zu Wien, 1883, lxxxvii, pp. 201–236; Grundlinien zur Erforschung des Helligkeits- und Farbensinnes der Thiere, Prag, 1884; Sitzungsberichte der kaiserl. Akademie der Wissenschaften zu Wien, 1885, xci, pp. 129–150.

intensity as well as of different wave lengths. But he, too, ignored entirely the third and most important factor, the *direction* of the light rays.

In the mean time a great deal of work had been done by Cohn, Engelmann, Wiesner and others on plants. Most significant of all was the paper published in 1878 by Strasburger.[1] He had experimented on many different kinds of swarm spores, and reached the conclusion that the movement of an organism in response to light takes place, in the majority of cases, in the direction of the rays. Under the influence of light, he maintains, an organism so orients itself that its main axis is parallel to the incoming rays, and the subsequent direction and definiteness of its movement towards or from the source of light are determined by the intensity of these rays.

This explanation of the reactions of these motile plant spores as purely mechanical phenomena was so simple and so reasonable that it seems strange that for twelve years no one applied it to animal forms. Bert, Lubbock, and Graber had all found it necessary to introduce a psychic element in order to explain their results, and it was left for Loeb[2] to show that the laws which Strasburger formulated for plant movements are the same as those which govern animal reactions. An animal, he states, so orients itself that its median plane lies in the direction of the impinging rays, and its movement is therefore in this direction. The precision of its orientation varies with the intensity of the light. "Light of unvarying intensity acts on animals as a constant heliotropic stimulus. The dependence of animal movements on light is point for point the same as the dependence of plant motion on this source of stimulation."[3]

The older idea of a response more or less psychical survives in a measure in the school led by Verworn and Oltmanns. They maintain that all light reactions are the result of an effort on the part of the organism to reach a certain optimum intensity. This explanation

[1] Strasburger, E.: Wirkung des Lichtes und der Wärme auf Schwärmsporen, Jena, 1878.

[2] Loeb, J.: Verhandlungen der physikal.-medicin. Gesellschaft zu Würzburg, 1888, pp 1–5; Der Heliotropismus der Thiere und seine Uebereinstimmung mit dem Heliotropismus der Pflanzen, Würzburg, 1890; Archiv f. d. ges. Physiol., 1890, xlvii, pp. 391–416.

[3] "Das Licht wirkt bei constanter Intensität dauernd als heliotropische Reizursache auf die Thiere. . . . Die Abhängigkeit der thierischen Bewegungen vom Licht ist Punkt für Punkt die gleiche wie die Abhängigkeit der pflanzlichen Bewegungen von derselbe Reizursache."

is, it seems to me, made improbable by the more careful investigations of Strasburger, Loeb, Driesch,[1] and others. Certain observations also which I have made lead me to agree with Loeb. One point, I believe, I have been able to add, namely, that the external conditions of light intensity and temperature remaining constant, other factors may, in some cases at least, intervene to change the character of the response.

The experiments to be described were made in the Biological Laboratory at Bryn Mawr College during the winter of 1898–99, under the direction of Professor T. H. Morgan, to whom I wish to express my indebtedness for kindly suggestion and criticism.

I decided to investigate the problem of light response in Ostracods, as little work had been done on this group, and *Cypridopsis vidua* var. *obesa* was chosen, because it was found to be more sensitive than any other species that could be obtained. It was also large enough so that the movements of isolated individuals could be followed. The experiments may be classified under three main divisions: —

I. Experiments in gaslight.
II. Experiments in diffuse daylight.
III. Experiments in direct sunlight.

I. Experiments in Gaslight.

Preliminary experiments were made with large numbers of individuals, brought from the aquaria into round shallow glass dishes which were painted black inside, in order to eliminate all possibility of reflection. But the results were so irregular and indefinite that it was found necessary to adopt some other method.

The apparatus finally decided upon was as follows: a tin trough (Fig. 1, A), painted black inside, ten inches long, $\frac{1}{2}$ inch wide and $\frac{1}{4}$ inch deep; a gas lamp B, Argand or Welsbach, placed 12 inches from the nearer end of the trough, and at an elevation of $6\frac{1}{2}$ inches, the arrangement being similar to that used by Davenport[2] in his experiments on Daphnia. Between

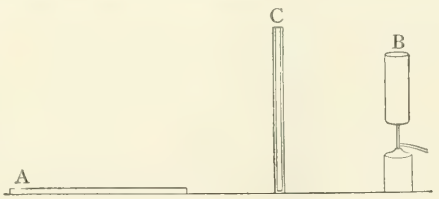

Figure 1.

[1] Driesch, H.: Zoologische Jahrbücher, 1898, v, p. 147.

[2] Davenport, C. B.: Experimental Morphology, Part I, New York, 1897, p. 204.

the lamp and the trough was fastened a screen C, consisting of a thin glass case containing water which was changed frequently. The entire apparatus was then placed under a hole in the ceiling, connected with a fan, by which the heat radiated from the lamp was drawn away, thus preventing any warming of the air in the room and consequent slow rise in temperature of the water in the trough. Black screens were placed around the trough to cut off the diffuse light of the room. Observations on the temperature of the water in the trough before and after the experiments showed usually that it varied very little.

Animals were taken from the aquarium and placed in glass evaporating dishes, from which they were to be moved one by one by means of a pipette into the trough. But I observed that if the individuals to be used stood exposed to the gaslight while others were being experimented on, they seemed later to have lost much of their sensitiveness. It became necessary, therefore, either to bring them in one by one from outside the dark room, or to keep them in a dark dish, taking out one as needed for the experiment. These two methods gave similar results, and the latter was chosen as more convenient.

Three different intensities of gaslight were used: the first, an Argand lamp turned low; second, the same raised to its full height, and third, a Welsbach lamp turned high. These were approximately in the proportions of 1:2:8.

The method of experiment was as follows. The trough was filled with water from the aquarium and its temperature recorded. A Cypridopsis was then taken with a pipette from the black dish and dropped into the middle of the tin trough. The direction of its first response, toward or from the light (+ or −), and the number of seconds taken for it to reach one or the other end, were noted. It was then given sixty seconds in which to change its position. If it remained at the end reached during this time, it was removed and the experiment repeated on another individual. If, however, it left this end before the sixty seconds passed, the length of its stay there, the character of its next response, + or −, and the time taken in any further reactions were noted. If in any case the individual showed no definite response, not reaching either end but wandering aimlessly about or remaining quiet in the middle of the trough, this was indicated by a ? or o. To illustrate, I copy the following series of observations from my note-book: —

Temp. 19° C.

Time.	No. of Experiment.	Sense of Response.	No. of secs. to reach end.
12.50 P.M.	1	+	35″
	2	0.+	80
	3	+	55
	4	+	50
	5	+	30
	6	+	35
	7	−	65
	8	+.0	
	9	−	25
	10	+ −	50, 40
3.50 P.M.	1	+ − +	20, 65, 70.
	2	+	25
	3	+	37
	4	+	25
	5	+	25
	6	+	60
	7	0	
	8	+	35
	9	0	
	10	+?	

In this way a very large number of observations were made, about eighty for each light intensity. Averages are given in the next table.

No. used.	Light intensity.	No. +	% +	No. −	No.[1] ±	% ±	No. ? or 0.	Average time.[2] +	Average time. −	% of ⊥. 1st +.	Longest time. +	Longest time. −	Shortest time. +	Shortest time. −
85	1	42 =	49 %	18	20 =	23 %	5	43″	40″	60	100″	100″	20″	20″
80	2	42 =	52 %	6	25 =	31 %	6	35″	45″	48	90″	60″	20″	25″
80	8	34 =	42 %	8	29 =	36 %	9	36″	45″	86	70″	80″	15″	18″
245		118 =	48 %	32	74 =	30 %	20							

[1] Individuals which changed from one response to the other. [2] That is, time taken to go from one end of the trough to the other.

In most cases the animals had been in diffuse daylight, as the aquarium was kept by a west window into which little sun found its way in the winter. But in the earlier experiments a careful record of previous conditions was not kept.

Examining these averages a few points are to be noted.

1. In general the time of response is shorter in the stronger light, though the difference is too slight to warrant one in drawing any inferences from it.

2. The proportion of those giving no definite response (?) or no response at all (0), is comparatively small.

3. The number of those giving both responses (±), and of these the proportion in which the first response is positive, is large.

The thought suggests itself that if the positive individuals had been left long enough, they too might subsequently have given a negative response.

The significance of these results being far from clear, further experiments were made to see if any clue could be obtained as to the cause of the irregularities. Apparently the same individuals could be now positive, now negative.

In order to determine whether the response of an individual was due either to the time of day at which the experiment was made, or to a definite period in its own life, a num-

ber were experimented on as above, and sorted into three dishes, +, −, and ±. At a different time on the following day these were tested again and others added. These again were re-sorted the next day and so on for several days in succession. But it was found that the results were quite irregular. Those with a given response at one time might give any other when tried again, though in the majority of cases here as before, the response was positive, or positive followed by negative. It thus became evident that the reaction of any one individual was not constant, but might vary at least from day to day. It might be objected that in dropping the animals into the trough, I unconsciously gave them an impetus in one direction or the other; but this error was carefully avoided either by dropping them in vertically or by putting the end of the pipette under water and letting them swim out as they would.

The next experiments[1] were made to determine whether the response of an individual was affected by merely picking it up. A Cypridopsis was taken from the dish with a pipette, dropped into the trough, and its first definite response noted. It was then picked up and again dropped in; this was repeated a number of times and each response noted. I cite one such series of observations: —

1. + + + + +	6. + + + + +
2. + + + + +	7. + + + + +
3. + + + + +	8. − + + + +
4. + + + + +	9. + + + + +
5. − + + + +	10. + + − − + − − + + + +

In almost all cases the first response was positive and where doubtful at first it became steadily positive after the animal had been several times disturbed. These results suggested the following series of experiments which served to harmonize some of the inconsistencies at first noticed.

The same apparatus as before was used, with the addition of another water screen at the opposite end of the trough.[2] The method

[1] In these and all following experiments in the dark room, the Welsbach lamp was used.

[2] The range of temperature within which the following observations were made was from 15° to 19° C.

was to drop a Cypridopsis, as before, into the middle of the trough and to note its first response as soon as well defined, but before it reached the end of the trough; the light was then quickly changed to a corresponding position at the other end, being moved directly over, and in a line parallel with the trough. If the animal turned in its course when the light was moved, its response remained positive or negative as before; if it continued its course *without change*, its response was noted as the opposite of that preceding. In this way the light was changed a number of times without the Cypridopsis ever being allowed to reach the end of the trough. After some time it was permitted to reach the end and to swim about as it would. If then it turned and started down the trough, in a direction, with regard to the light, *opposite* to its former course, the lamp was again moved as before to determine whether this new direction of movement represented a distinct reaction to the light, or whether it was purely accidental. In almost all cases a very definite result was obtained; the first response was retained for some time by constantly shifting the light, the animal turning with each change and never touching the end of the trough. If then it was allowed to reach the end and to swim about there, it sooner or later turned and moved down the trough in the opposite direction. If the lamp was again shifted from end to end, this new response — *the opposite of the preceding* — persisted in a similar manner. If, for example, the Cypridopsis was at first positive, *i. e.* moved toward the source of light, — and this was generally the case, — if the lamp was moved the animal turned about in its course and swam again toward the light; if moved again, it turned again, and so on, *provided always that it was never given time to reach either end.* If now it was allowed to come to the end and left to swim about there, sooner or later according to the individual, and in a certain degree according to previous conditions,[1] it turned about and moved down the trough *away from the light.* If then the light was shifted from end to end as before, this negative response persisted, as had the positive before. If, finally, the negative end of the dish was reached, in the same way a subsequent change to positive followed.

In a few cases the change from positive to negative (never from negative to positive) occurred soon after the animal was dropped in and before it touched the end. But such instances were rare. Usually the change from positive to negative took place in less time than that

[1] See pp. 358 and 363.

from negative to positive,[1] so that if a number of individuals were put in the dish at once, there was a gradual accumulation at the negative end. The changes from positive to negative and back again could be repeated any number of times on the same individual. The ± individuals in the earlier series of experiments were simply those in which the first change took place in less than sixty seconds.

In this same connection another phenomenon was observed. It has been suggested that the stimulus of picking up the animal may be one factor in causing its first positive response. It was found that if an animal in the negative condition, if we may so call it, *i. e.* in its course down the trough away from the light, was picked up with the pipette and dropped in again, either in the same spot from which it came or in any other part of the trough, it became positive, *i. e.*, it moved toward the light. In the majority of cases this change took place after once picking up, although sometimes an individual was taken up a number of times in succession before a permanent change was effected, that is, before a distinct positive response followed.[2] Different sized pipettes were tried, but the same result was obtained. It was also found that sufficient agitation of the water alone would make the individuals positive, but in no case did I succeed in making a positive animal negative in this way. If the animals be shaken when brought to the light, the very great majority are at first distinctly positive, though many soon become negative.[3]

In seeking an explanation for these phenomena, one idea that suggested itself was that as the trough was made of tin and the ends cast a small shadow, the change at the ends might be due, in part at least, to the influence of the shadow. To obviate this difficulty pieces of cover-slips were placed obliquely at the ends in such a way that the animals could not get into the shadow. The Cypridopsis then swam to the surface of the water, up the incline of the glass, but the same changes took place as before. I tried also screening parts of the trough from the light, but found that the shadows alone did not change the character of the response, though if they were long enough they

[1] This latter change occurred only after long intervals in the case of Cypridopsis which had been for some time previously exposed to the sunlight. In these the positive condition, if found at all, was always of brief duration. (See p. 358.)

[2] This was most noticeably the case when the animals were brought from exposure to direct sunlight.

[3] A similar phenomenon was observed by Loeb (Archiv f. d. ges. Physiol., 1893, p. 96), in the case of Copepods, but he did not attempt to explain it.

checked the response altogether. Moreover, the change from negative to positive takes place always in the end of the trough in which there is no shadow.

Again, this change might be due to something in the character of the ends themselves. Wedges of cork were therefore substituted which also cut off the shadow, but they did not affect the results. And, finally, the same results were obtained when the animals were placed in a prism containing water and so tipped (Fig. 2) that the water made its own edge at x, in contact with the air and the bottom of the prism. Here too, a Cypridopsis could be driven up and down the sloping bottom by shifting the light, while it changed as before from positive to negative or the reverse if left alone.

FIGURE 2.

That the phenomena were in no way due to differences in intensity of light, was proved by placing over the trough a prism with a glass bottom (Fig. 3) similar to that used by Strasburger [1] and Davenport,[2] and containing a weak solution of India ink in water. The animals were dropped into the trough and the light shifted from end to end as before, while the prism was not moved. The positive individuals moved always toward the source of light, going either to the lighter or darker end, as the case might be. If the light remained stationary for a time at either end, the characteristic change from positive to negative took place in some of the individuals. If the light was again shifted, the remaining positive animals followed the light as before, while the negative ones moved always away from it, into either the darker or lighter end. This made it clear that even considerable variations in the intensity of light at the two ends had no power to change the response.

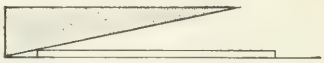

FIGURE 3.

Loeb's [3] experiments with Polygordius larvæ and Copepods suggested that the change might be due to small variations in temperature. But readings taken at different places in the trough showed it to be uniform throughout.

Another possible explanation of the changes observed was that the position of the light above the plane of the trough and the angle at which the rays entered it might in some way affect the response. To

[1] STRASBURGER: *loc. cit.*, p. 35. [2] DAVENPORT: *loc. cit.*, p. 204.
[3] LOEB: Archiv f. d. ges. Physiol., 1893, pp. 90–96.

avoid this possibility I used another trough exactly similar to the first, except that the ends were of glass. I then placed the light so that the rays entered the trough perpendicular to its ends, and parallel to the surface of the water inside. These experiments showed only that in some cases the time required for the change was longer, but even this was uncertain. It also seemed to me possible that if the trough were longer so that the animal would have to swim a greater distance before touching the end, it might change its course, and hence the character of its response, before the end was reached. To determine this I used a longer trough, 18 × 3 × 3 inches, painted black inside, and with ends of glass like the smaller one. But in this the same results were obtained. One Cypridopsis would swim the entire length of the dish, showing no sign of turning, would reach the end and swim about there for more or less time, then suddenly turn and swim back. And in both cases it would respond, as before, to changes in the position of the light. In fact, this change of response seemed to be characteristic of the animal, for I met it under all circumstances, in all kinds of dishes, and in all intensities of light. There was a constant interchange of positive and negative individuals, each one, for the time being, perfectly definite in its reaction, and ready to follow any shifting in the position of the light. And it was this characteristic that had made my earlier results so irregular and inexplicable, and which could have been discovered only by observations of isolated individuals.

Davenport[1] in his experiments with Daphnia, dropped several individuals at once into a trough, and after a given time, let down a series of partitions which divided the trough into three parts. He then counted the number of individuals in each of the cells so formed, and found that the greater number of Daphnia had moved toward the light. That the usual response of Daphnia is a positive one, there can be no doubt, and yet I have found while working with Cypridopsis, individual Daphnia as markedly negative as the majority were positive; and the question arises whether they too might not, under certain conditions, show a similar change of character. It is evident that had Davenport's method been followed in my earlier set of experiments, and a time limit of sixty seconds been used, the results would have pointed strongly to Cypridopsis as a positive form; while later experiments show that with a longer limit, exactly opposite conclusions might have been reached.

[1] Davenport, C. B., and Cannon, W. B.: Journal of Physiology, 1897, xxi, p. 26.

I have found in the literature of the subject only two mentions of similar phenomena. The first was made by Strasburger [1] with reference to Ulothrix swarm spores. He says:[2] "On the positive as well as on the negative side individual swarm-spores may constantly be observed to leave the margin suddenly and hasten straight through the drops to the other margin. This exchange between the margins takes place uninterruptedly." This behavior he explains later, saying that the spores were then probably exposed to nearly their optimum intensity, and that therefore it was indifferent to them whether they moved toward or from the light, so long as their path lay parallel to the rays. That this explanation does not apply to Cypridopsis appears from the fact that the same phenomenon is met with in a wide range of intensities, not only of gaslight but also [3] of diffuse light and direct sunlight.

The second observation of this kind was made by Groom and Loeb [4] on Nauplii. After exposure to light they become negative, but if covered for a few moments and then again exposed, they are temporarily positive. Loeb maintains that the character of the response is modified by the preceding intensity to which the organism has just been exposed. But it is hard to believe that in the case of Cypridopsis a short exposure to the intensity of light at the positive end makes it negative, and a similar exposure at the negative end makes it positive. For (1) in the shorter trough with parallel light, the intensity can differ but little at the two ends; (2) the change takes place no sooner in the very long trough than in the short one; and (3) when the trough is covered with an ink prism the same changes take place *at either end*.[5]

The problem, therefore, which presents itself is as follows: Cypri-

[1] Strasburger: *loc. cit.*, p. 17.

[2] "Ununterbrochen bemerkt man auch, sowohl an der positiven als auch an der negativen Seite, einzelne Schwärmer, die plötzlich den Rand verlassen und gerade aus durch den Tropfen nach dem anderen Rande eilen. Ein solcher Austausch findet ununterbrochen zwischen beiden Rändern statt."

[3] See pp. 359 and 363.

[4] Groom, T. T., and Loeb, J.: Biologisches Centralblatt, 1890, x, pp. 171–2.

[5] It is worth noting in this connection that in the spring of 1898, while experimenting on some small Phyllopods, I observed a somewhat similar phenomenon. When the organisms were first taken from the aquarium to a glass evaporating dish and set before the window, they gathered in swarms on the negative side of the dish, but if left undisturbed for some time they moved over to the positive side. If the dish was again shaken, they again swarmed on the negative side, to return as before when left for a time. No explanation for this behavior offered itself.

dopsis vidua, when exposed to light, undergoes successive changes in response from positive to negative, from negative to positive. The possible factors determining these changes may be (1) internal, (2) external, or (3) both working together. Of the external factors, variations in light intensity and in temperature may be eliminated. The one remaining which suggests itself most obviously is contact, and my first conclusion was that this was the cause of all the phenomena. This view was strengthened by the following experiment.

A maze of seventy-five very fine sewing needles (No. 12), was arranged in the middle of the small trough, and it was placed in front of the light as before. A Cypridopsis was dropped into the farther end, and its course watched. As the needles were set very close together, the animal in its course toward the light had to thread its way among them, and came into forcible contact with a number in succession. In the majority of cases, the response of such an individual changed from positive to negative in its first course through the needles, and it often turned before it had found the end of the maze. A few animals wandered for some time among the needles without finding their way out, and then usually came out from the end which they had entered, being then negative in response. In these experiments also, the positive individuals changed more quickly to negative than did the negative to positive. That contact may play a part in the transformation was thus made clear. But upon more careful consideration I saw that it failed to explain all the results satisfactorily, and in October and November, 1899, I made a somewhat closer study of the problem.

In the first place it was evident that the term "contact" must be used in a broad sense to cover not only the forcible hitting of the animal against the ends of the dish, but also the mechanical stimulus which it receives when picked up or shaken in the water. But this latter stimulus acts always to change a negative animal to positive, never a positive one to negative, while contact with the end apparently effects both changes. Have we therefore two entirely different factors at work here? Or is one but a more or less intense form of the other? Or, finally, does some third element enter in? If contact is the sole cause why should the positive reaction be changed to negative sooner than the negative to positive? And again, why should the same individual change with greater ease at one time than at another? I found that an animal that had just become negative, and had therefore just left the positive end, was generally more

easily changed to positive than one which had progressed for some distance down the trough toward the negative end.

It seemed to me, therefore, that the important point remaining to be determined was whether the change in response could take place independently of external stimuli; or, in other words, whether the change was due entirely to the working of external factors or might result wholly from internal causes. For this purpose I used the larger trough, dropping the animals in as before. My purpose was by constantly changing the position of the light, to keep the Cypridopsis from touching either end at any time; and to see whether in this case, contact being eliminated, the usual change in response would occur. In nearly all cases the result was as follows: Each animal when first dropped in was positive; by shifting the light I was able to keep it so for varying lengths of time — dependent to a certain extent upon the intensity to which it had been exposed previous to the experiment (*cf.* pp. 353 and 363). Sooner or later, the average time being from two to five minutes, the positive response ceased and was followed sometimes by a period of torpidity, due possibly to fatigue, in which no change in the light produced any effect whatever. After this torpid period or without it, the negative response began. And in all cases this persisted, as long as I continued to shift the light, or until the animal was evidently fatigued. One individual I kept negative for half an hour in this way. But I was able at any time, by more or less agitation of the water or by picking up the Cypridopsis, to induce a temporary positive response.

In order to assure myself of the truth of these results I repeated the experiments in a trough 8 feet long and 7 inches wide. In this the light needed to be moved much less often, and so possible confusion was avoided. *In all cases the positive response was temporary*,[1] *while the negative one persisted as long as the animal could be kept in motion.* In one case I held the light over the middle of the trough. The animal would stop and swim about under it for more or less time and then turn and swim back as it had come.

These final experiments make it clear, I think, that the normal response to light of Cypridopsis vidua is not, as would at first appear, positive. The positive condition is, on the other hand, only a temporary one, induced by some mechanical stimulus, such as contact, jarring, etc., and sooner or later, according to the intensity of

[1] The duration of the positive response was shorter when the Cypridopsis had been previously exposed to direct sunlight.

light to which the organism has been for some time exposed, it gives place to the more stable negative condition.

Positive and negative responses, then, represent different phases in the internal condition of the animal. Positive changes to negative, independently of external conditions, after the lapse of a varying length of time; negative gives place to positive much less readily, and only when induced to change by the action of some mechanical stimulus. In the latter external factors are the more immediate cause, while in the former only internal conditions operate.

II. Experiments in Diffuse Daylight.

These experiments were made in order to ascertain whether the phenomena described before were to be met with only in artificial light (much weaker than that normal to the individuals), or whether the reactions were also characteristic of the form under normal conditions. For this purpose the smaller trough was placed close to a window where it received only diffuse light. All light except that coming directly from the window was cut off by means of black triangular screens. The bottom of the trough was marked into thirds, and four or five animals were dropped into the middle third. A series of observations was then taken, every half minute or every minute, of the number of individuals in each third. The results when averaged were as follows. Only the first set of observations and one after from eight to ten minutes are given: —

Interval in minutes.	+ Third.	Middle.	− Third.	Total	Remarks
0.5–1.0	82 = 59%	39 = 28%	18 = 13%	139	
8–10	31 = 22.6%	30 = 21.9%	76 = 55.5%	137	2 lost.

From this table it will be seen that the first response in the majority of cases was positive, and that later there followed an accumulation of individuals in the negative end. If the experiment was continued longer, there was a constant interchange between the two ends, but the greater number of animals were found in the negative end. If an evaporating dish was left in the window for some time, properly screened from the room about, the majority collected on the negative side, but there was always a certain amount of interchange as above.

III. Experiments in Direct Sunlight.

This series of experiments has no direct connection with those given above, but was suggested by Oltmanns' paper[1] of 1892. Oltmanns worked with a large number of plant forms, motile and others, *Volvox globator*, and *V. minor*, *Spirogyra*, *Mesocarpus*, *Vaucheria*, etc The experiments on Volvox are the ones to which I wish to direct especial attention.

Oltmanns used a box, two opposite sides of which consisted of prisms of gelatine containing India ink. The dish containing Volvox was put under this box and the latter placed before a window as in Fig. 4, so that the sunlight fell on one end at an upward angle but parallel to the sides. The brightest region was thus at A, the darkest at B; but, he argues, the regions of equal intensity lay, not in planes vertical to the surface of the water, but at more or less of an angle with it toward the position of the sun.[2] Under these conditions he found that the parthenogenetic individuals collected in great numbers in the brightest part, while the females arranged themselves in rows which were longer at the darker end, at a depth corresponding, as he believed, to their optimum intensity. He then placed the box with the sun shining on the prism from an elevation of 45° but parallel to the closed ends. Under these conditions all left the brightest region, the females gathering in the darkest, and the parthenogenetic individuals in a somewhat lighter part. If a cloud covered the sun, all moved further toward the clearer end. In no instance, he says, was there any sign of movement in the direction of the light rays. From these and other experiments Oltmanns concludes that every organism is attuned to a certain intensity of light, and that all its responses to light stimuli result from attempts to reach this optimum. The influence of ray direction is only secondary. An organism may move toward the source of light and parallel to its rays, but it does so because, by so doing, it is approaching more nearly the intensity which is its optimum. But Olt-

Figure 4.

[1] Oltmanns, F.: Flora, 1892, lxxv, pp. 183–266.

[2] The argument is not at all clear, but this is, I think, what is meant.

manns' experiments on Volvox were inadequate to prove his point. For in the first experiment, in which the sun's rays fell at an angle on a closed end of the trough, and parallel to the sides, only diffuse light of uncertain direction could penetrate to the interior, and therefore the exact position of planes of equal intensity could not be accurately determined. In the second experiment, the angle at which the direct rays entered, made accurate results less possible; but apart from this, as my experiments will show, the conditions were not as simple as they seemed.

It was my purpose, therefore, to modify Oltmanns' method in such a way as to avoid possible objections and to determine whether such forms as Cypridopsis or Daphnia would evince any preference for a definite light intensity, or whether the direction of movement might not depend solely on the direction of the rays of light.

The apparatus used was a tin box 12×9×5 inches, with one end of glass and a close cover. The entire interior, excepting the glass plate, was painted black. Over the glass end I used an India ink prism, varying the thickness and intensity at different times. On this prism the sunlight was reflected by means of a mirror, so that the rays entered either perpendicular to the prism or at an angle with it, as desired, but always parallel to the surface of the water within.[1] If the sunlight fall perpendicular to the prism, in theory there should be in the water parallel planes of light of regularly decreasing intensity, corresponding to the gradation in the prism (AA′ to BB′, Fig. 5), that is, no light should reach A′ except that which has passed through A, or B′ except that which has passed through B. But that this is not the case may be easily seen, for there are of necessity innumerable particles in the water which will diffuse light, for example, from B to A′.[2] The conditions are therefore not so simple as they at first appear. An

FIGURE 5.

[1] In the later experiments a glass plate was placed in the water at about half its depth, and the animals were dropped on this, so that any shadow from the front edge of the trough falling on the bottom might be avoided, and also that the diffuse rays from above might be balanced by similar ones from below.

[2] This can be demonstrated by making observations through an aquarium of which A′B′ is also of glass. The light from B is very distinct and an important factor in the illumination of A′

organism placed at A′ may be influenced by (1) the *direct* light which passes through A, and (2) the *diffuse* light coming from B. The relative value of these two forces with respect to the organism will therefore determine their resultant effect, and hence the direction of its response. If a Cypridopsis or Daphnia be dropped into the trough at A′ and be seen to move in a line which forms a distinct angle with AA′, and toward B, nothing can be inferred in regard to its response to light *intensity*, but we may only conclude that the light from B, as well as that from A, is exerting a sensible effect upon its movement. Even this diagonal course, therefore, may be determined by the *direction* of the rays impinging upon the organism. This theory was, I found, confirmed with large numbers of Cypridopsis on which I worked, and even more distinctly with Daphnia, which are more sensitive to slight variations in the direction of the incoming rays. Individuals were brought from diffuse daylight, in which, as has been seen, their first response is positive, and placed for a few minutes in the black dish used in Experiment I. This treatment made their subsequent response somewhat more sensitive. They were then dropped at various points into the box. In the majority of cases a *distinctly diagonal course was taken.* If a black screen was placed in front of the lighter end of the prism, so cutting off the strong diffuse rays, the path taken became parallel to the (stronger) direct rays; on removing the screen the diagonal course was resumed. In the case of some Daphnia this operation could be repeated several times as they moved down the box, their direction changing very quickly with changes in the light. The entire course of one may be represented by the line in Fig. 5. With a Cypridopsis only one or two such changes could be effected in one trip from end to end.

To prove that the light from B was the factor which caused these changes in direction, the screen was interposed over the darker end of the prism A, thus cutting off from the organism all direct rays; a still more distinctly diagonal course resulted. If a Cypridopsis or Daphnia was dropped in at B′ and B covered, a diagonal movement toward A followed, but if B remained uncovered, the course taken was parallel to the direct rays, the very weak diffuse rays from A having no appreciable effect in altering the course of the animal.

If the conclusions given above be correct, it should be possible to cause Cypridopsis or Daphnia to move perpendicular to the lighted end of the trough by reflecting the sun's rays into the trough at an angle with the prism, as shown in Fig. 6, for at a certain angle the

resultant movement of the two forces should be in the direction A′A. In this case, part of the box is in shadow and care must be taken to drop the animals outside of this and within the influence of the direct rays. *The anticipated result was obtained with both Cypridopsis and Daphnia.* At a certain[1] angle of the direct rays with AB the individual moved in a line parallel to the sides of the trough; if the angle was increased a corresponding bending of the course toward A was noticeable.[2] (For these more delicate reactions Daphnia were found more satisfactory, for the angle of the rays had to be much greater to induce the Cypridopsis to move into a region of less intensity.) If either a Cypridopsis or Daphnia was dropped into the part of the dish which was in shadow, *i. e.*, into which no direct rays found their way, it moved diagonally toward B until it came under the influence of the direct rays, when its course was altered as above stated.

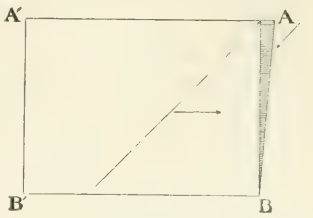

FIGURE 6.

Finally, I believed that the experiments would be complete if the reactions of a negative Cypridopsis could be observed, as its response should be the exact opposite of that of a positive individual. But there is the difficulty that when the organisms are brought from diffuse light, or from the black dish, into the trough, they are made distinctly positive by the process of transfer; and those which are left in the trough gradually collect in the light region, moving parallel to the side from the positive to negative ends in characteristic manner. Neither of these groups of individuals is available therefore for the purpose. But I found that after long exposure to direct sunlight Cypridopsis became so strongly negative that it was very difficult to change it by any amount of disturbance. It proved to be strongly negative when brought into gaslight, also in diffuse light and in sunlight that had passed through the prism. Such negative individuals when dropped into the box at B, responded as expected, moving in a diagonal course toward A′, if the light was perpendicular to AB, and parallel to BB′ when the light was at an angle toward A.

[1] Dependent on the intensity of the light and the thickness of the prism.

[2] In the dark room experiments in which the prism was used, the direct rays formed such a great angle with the diffuse that the influence of the latter did not appear.

Dropped within the edge of the shadow a negative Cypridopsis moved away from the strongest light, namely, in the direction BA'; while a positive Daphnia dropped in at the same time, moved in an exactly opposite direction.

Davenport,[1] in his "Experimental Morphology," makes the provisional statement that "if two rays of different intensities making an angle with each other, fall upon the organism, it apparently moves in the direction of the intenser ray, if free to do so." That this is not the case in this form at least, has been seen. Each ray exerts its influence independently of the other, and the direction of the resultant movement of the organism is conditioned by the direction and the relative intensity of the impinging rays.

In the November number of this Journal, there appeared an article by R. M. Yerkes on the "Reaction of Entomostraca to Stimulation by Light." In this the author endeavors to prove that the organism Simocephalus vetulus Mueller may give a photopathic reaction as well as the phototactic one which Davenport and Cannon proved for it in 1897. For this purpose he used an apparatus which was similar in principle to that used in my third series of experiments, except that the trough was proportionately much narrower, thus preventing, as he claims, all possibility of phototactic reaction. The light, first diffused, passed through an ink prism into the side of the trough in a direction perpendicular to its long axis. The trough was divided into compartments and in them certain individuals were placed. The partitions were raised after a certain time, three minutes being most favorable to the desired result. Yerkes found that at the end of this time the majority of individuals had gathered in the positive (lighter) end of the trough. He concludes therefore that "Simocephalus vetulus Mueller is positively (+) photopathic." This is, I believe, another instance of an erroneous conclusion being drawn from the results of experiments with a prism. *To an individual Simocephalus* placed in the darker part of the trough, the source of light is the lighter end of the trough, and toward this it moves. The "source of light" is, it seems to me, only another name for the point or region from which the most intense rays come; and when an organism, placed in the conditions created by Yerkes, moves toward the lighter region, it is only moving in the path of the most intense rays that strike it, and is then still phototactic.

[1] DAVENPORT: *loc. cit.*, p. 208.

Another point to which I should like to call attention is the interesting variation caused by changing the time of the experiment. Three minutes gave the maximum positive response (and was therefore chosen as the most favorable period), while "when the time became longer than three minutes, the result showed a decrease." "It seems probable," says the author, "that during the first two minutes all the animals have time to move to the most intensely lighted portion of the trough, *i. e.*, space 1. After moving about for a few seconds there they begin to move in the only direction possible, namely, toward the negative end of the trough. Whether this is due to a gradual lessening of the stimulation is uncertain. It might well be that after a few trips back and forth most of the animals would come to rest in that intensity of light most suitable for them, — that to which they are 'attuned.'" This view is not maintained by experimental evidence and seems to me improbable. I should like to suggest that the true explanation may be found in some such phenomenon as that which I have described in the case of Cypridopsis vidua.

Summary.

I. The condition of an individual of the species Cypridopsis vidua, with reference to its reaction to light, changes, under constant conditions of temperature and light intensity, from positive to negative, from negative to positive. The former change is brought about solely by internal causes, while the more immediate cause of the latter is an external one, namely, mechanical stimulation.

II. The direction of movement of Cypridopsis and of Daphnia in response to light does not result from an effort on the part of the individual to reach a certain optimum intensity. It is determined (1) by the directions of all the impinging rays, and (2) by the relative value of these rays as forces acting upon the organism, *i. e.*, by their relative intensities. The resultant direction could be found by compounding all these forces if their direction and relative value were known.

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THE STRUCTURE OF THE EYE OF SCUTIGERA (CERMATIA) FORCEPS.

JOSEPHINE HEMENWAY.

GRENACHER ('80), in his article entitled "Ueber die Augen einiger Myriapoden," described the structure of the eye of Scutigera (Cermatia araneoidea). Briefly reviewed, his account is as follows:

Externally the eye of Scutigera has the appearance of a true facet eye, consisting of one hundred of these "facets." To each facet there corresponds an ommatidium. Each ommatidium consists of a central crystalline body, surrounded by three tiers of cells; the distal tier of pigment cells, the middle and proximal tiers of retinular cells secreting on their inner edges a narrow band, the rhabdom. The crystalline body is composed of very irregular segments. These segments may be either cells or cuticular structures. In the adult eye they cannot be regarded as cells, as nuclei are not found in them, although Grenacher admits that at some time in their existence they may have been cells, later becoming modified and losing their nuclei. The possibility of their being secretion products he does not admit, as he finds no cells to which their origin could be traced. There are six to eight or nine of these segments.

The retinular cells with their rhabdoms embrace the proximal two-thirds or three-fourths of the crystalline body, the posterior portion of the retinular cells reaching to the basal membrane. Of the three tiers of cells surrounding the crystalline body, the middle tier, or outer retinula, is made up of from nine to twelve cells; the proximal tier, or inner retinula, of three to four cells. Sections through the proximal layer show that at this level the rhabdom is made up of four parts. Toward the extreme proximal end of these proximal retinular cells only three with their rhabdoms are visible in cross-

sections, the fourth having been pushed out. The nuclei of the retinular cells lie in the distal portion. The pigmentation of the eye consists partly of the pigment granules in the retinular cells and partly in the separate pigment cells. Of the latter there are three distinct groups: (1) a circle of from eight to ten large flattened cells, the outer tier of my description around the outer part of the crystalline body; (2) long, spindle-shaped pigment cells situated between the ommatidia, extending to the inner cuticula; (3) a third group, the supplementary cells of my account, is found on the posterior part of the retinula, between the retinular cells.

Grenacher also mentions the pigmentation of the optic nerve and the "inner cuticula."

Adensamer ('93), in his studies on this eye (Scutigera coleoptrata), confirms and completes Grenacher's statements. He differs in certain points.

The cornea of each ommatidium Grenacher regarded as externally convex, although there were individual differences. These differences Adensamer regards as stages in the development of the cornea.

In the adult eye frequently there were found in the crystalline body large yellowish enclosures, which had the appearance of fat drops. These are not to be confused with the nuclei for which Grenacher looked. Of the segments he found from seven to nine.

But in an individual 5 cm. long Adensamer states that he found nuclei in the crystalline body; thus he feels justified in calling the segments "cells."

As to the nerve fibers he was more successful than Grenacher, in that he saw the connection of the fiber with the outer and inner row of retinular cells. This he proved by sections. Just under the basal membrane there is a nerve connected with a muscle, which is entirely distinct from the optic nerve. Adensamer believes that this was probably mistaken by Grenacher for the real optic nerve. The latter consists of a bundle formed of the separate nerve fibers meeting proximal to the basal membrane.

Speaking of the superficial resemblance of the eye of Scutigera to that of insects and crustaceans, and the actual differ-

ences between them, Adensamer suggests calling the eye of Scutigera a "pseudo-facet" eye. Rosenstadt ('96) discusses the question as to whether the eye of Scutigera can be regarded as a true facet eye, reviewing the arguments of Grenacher and Adensamer. He also suggests a way by which an eye, as that of Scutigera, could be developed from a true facet eye.

The following work was done in the Biological Laboratory of Bryn Mawr College, under the direction of Prof. T. H. Morgan, to whom I am greatly indebted for valuable suggestions and criticism.

The species studied was Scutigera (Cermatia) forceps.

For sectioning, the best results were obtained by hardening the fresh material in corrosive acetic for fifteen minutes, then running it up through the successive grades of alcohol.

The dense pigment obscured all details, therefore a depigmenting agent, as KOH, was used (*cf.* Parker, "The Eyes in Scorpions," '87). The preparations were stained with iron-haematoxylin.

As a maceration fluid, a modification[1] of Béla Haller's fluid was used. Material left in it for a year gave excellent results. The separate ommatidia fell apart, and by gently tapping the preparation the individual cells of each ommatidium could be isolated.

By this means I have been able to make out more definitely the structure of the different component cells than have the authors mentioned above, and in some respects have been able to add some points to their results.

The eye of Scutigera forceps is nearly triangular in shape. The corneal hypodermis is faceted, one ommatidium corresponding to each facet. Each eye is composed of about two hundred individual units or ommatidia.

Fig. 1 *A* shows[2] a single ommatidium, its proximal end bordering on the inner cuticula or basal membrane.

[1] Béla Haller's mixture modified: two parts glacial acetic acid; two parts water; one part glycerine.

[2] The figures are all camera drawings: Fig. 1 *A* and *B* were drawn with a No. 7 objective; Fig. 1 *C* and *D* were drawn with an oil immersion $\frac{1}{12}$; Fig. 2 *A*, *B*, *C*, *D*, *E*, *F* were drawn with an oil immersion $\frac{1}{12}$.

Surrounding each ommatidium are elongated pigment cells, extending the entire length of the ommatidium (Fig. 1 *B*). At both distal and proximal ends these pigment cells become expanded, the pigment granules collecting in the expanded portions. At the proximal end this gives the pigmented appearance of the basal membrane, spoken of by Grenacher.

There are sixteen to eighteen of these pigment cells belonging to an individual ommatidium. The nuclei are visible without reagents, but are more clearly shown by methyl green.

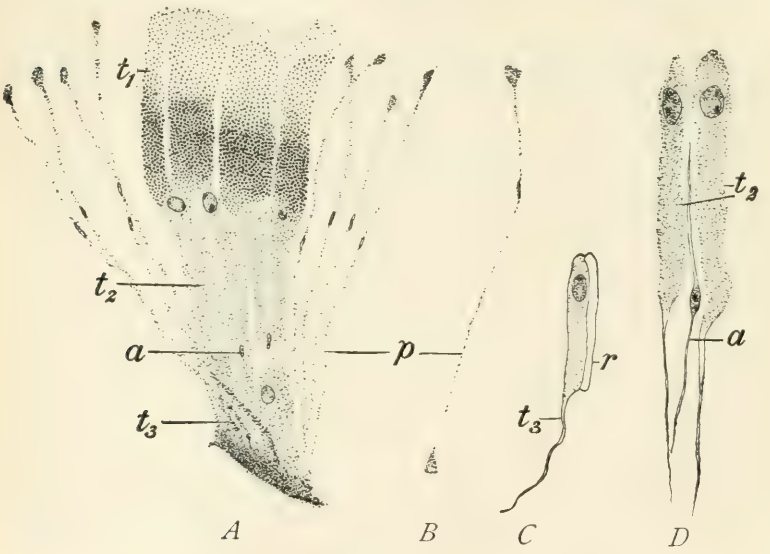

Fig. 1.

They are found at the same level as the nuclei of the middle tier of cells (Fig. 1 *A*). Each ommatidium consists of a clear, crystalline body, surrounded by three tiers of cells; the outer consisting of twelve (t_1), the middle of ten to twelve (t_2), and the inner tier of three to four cells, respectively (t_3).

The cells of the outer tier are large and flat and deeply pigmented at the proximal ends, the pigment granules being extremely large and round. The nuclei did not show in a macerated specimen, owing to the pigment.

The middle tier of cells are called by Grenacher the "outer retinular cells." They are longer and more narrow than the

cells of the outer tier, reddish in color, lacking the black pigment of the outer tier.

(Fig. 1) *D* t_2 shows two of these cells with the nuclei at the extreme distal portion. At the proximal end each cell is prolonged into an extremely fine "tail," which runs down between the cells of the inner tier and is continued through the basal membrane as a nerve fiber.

The cells of the inner tier have their proximal ends bordering upon the basal membrane. From the proximal ends fine processes continue through the basal membrane to form the nerve fibers. The cells are much broader than those of the middle tier.

Cross-sections through the different levels show the crystalline body occupying the central axis of the ommatidium, surrounded by a clear zone or rhabdom forming the inner parts of the cells of the ommatidium (Fig. 2 *B*, *r*). The clear zone is formed of the structures called by Grenacher the rhabdoms — a secretion product of the retinular cells. In macerated specimens these rhabdoms were visible upon the inner surface of each cell of the two proximal tiers (Fig. 1 *C*) and could be made to separate from the cell by tapping. The "tail," or nerve, is on the opposite side of the cell from the secreted portion. The secretions from the inner tier are thicker than from the middle tier, and in cross-sections appear roughly triangular in shape (Fig. 2 *F*). There were no nerve fibers observed for the outer tier, and it differs in this respect from the two inner tiers. In certain cases, after tapping, the outer cells unfolded, as it were, and spread out into a band. The distal ends are rounded, while the proximal ends are drawn down into a point which extends between the distal ends of the middle tier. Thus the nuclei of the middle tier are found between these points of the outer tier (Fig. 1 *A*).

The series of cross-sections (Fig. 2 *A–F*) are taken at the levels of the different nuclei.

I. The first section beneath the cornea is shown in Fig. 2 *A*. It represents the extreme distal portion of the ommatidia. A few large nuclei are found at this level, situated between the individual ommatidia.

II. The next figure (Fig. 2 *B*) is taken through the nuclei of the outer tier of cells, two sections intervening between *A* and *B*. These nuclei differ in shape from the round ones of the middle tier.

III. The next section (Fig. 2 *C*) shows two sets of nuclei, the larger ones (*n*) belonging to the middle tier of cells, the smaller (*p*) being the nuclei from the surrounding pigment cells (Fig. 1 *A*, *p*).

IV. Fig. 2 *D* shows a section through the nuclei of the middle tier of cells. This drawing is from the same section as *C*, but drawn at a lower level.

V. The following figure (*E*) is the fourth section after *D*. The round nuclei are from the clear cells or supplementary cells.

VI. Situated at about the same level as the nuclei shown in Fig. 2 *E*, but in the next section (Fig. 2 *F*), are the nuclei belonging to the inner tier of cells (Fig. 1 *A*, t_3).

The nuclei shown in Fig. 2 *E* belong to a group of cells lying between the two proximal tiers of cells. These cells are very different from any of the others found. They are colorless and contain no granules. Fig. 1 *D* shows two cells from the middle tier, between them one of these supplementary cells, in its natural position. The supplementary cells are extremely delicate, sending distally a fine process between the cells of the middle tier, and proximally between those of the inner tier. The nucleus is small and easily distinguished from that of a cell of the inner tier. The nuclei are found at the level of the distal ends of the inner cells. There are four supplementary cells.

The crystalline body is surrounded by the tiers of cells described above. It is composed of segments — "Grenacher's segments." These segments are cone-shaped. At the level of the nuclei of the inner tier (Fig. 2 *F*) these are not seen in cross-section, the rhabdoms, only, belonging to these cells being visible. In maceration preparations they often separate from each other at the proximal ends, while found joined at the larger distal ends.

According to Adensamer there are nuclei in these bodies

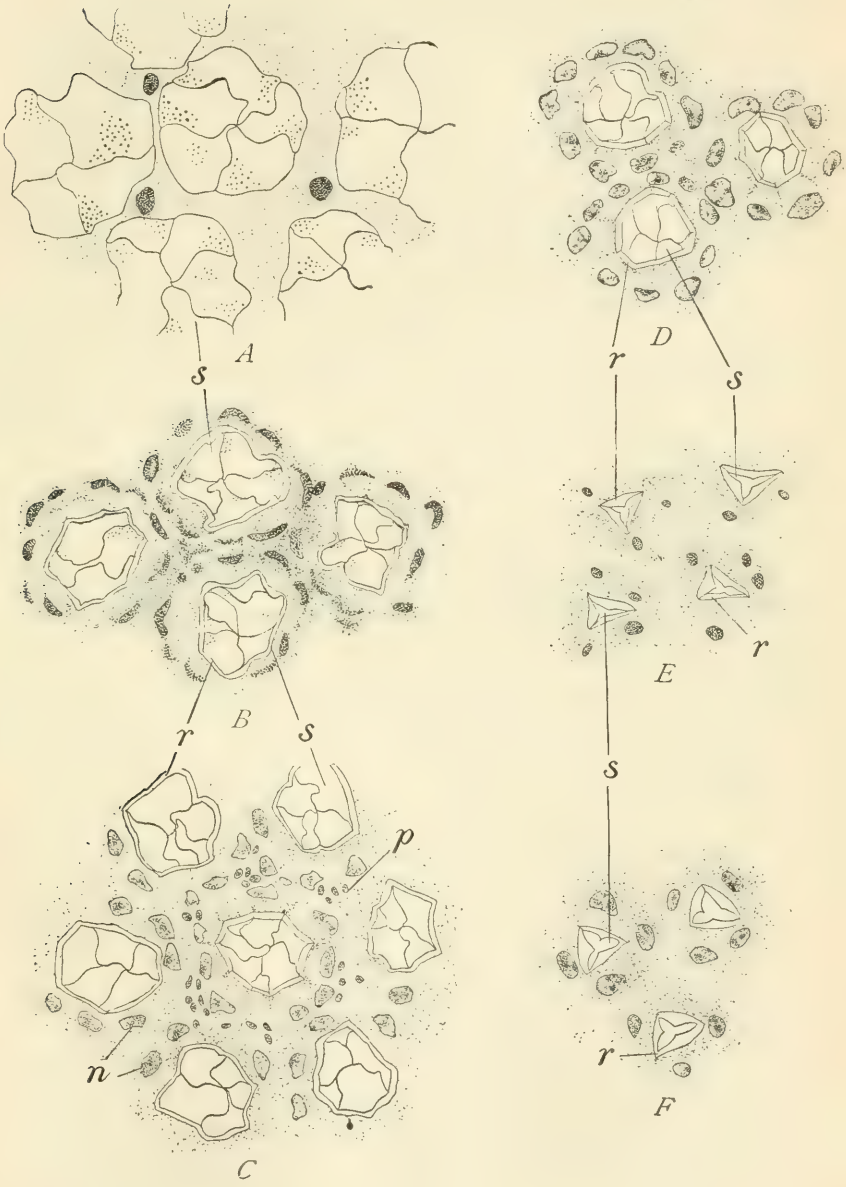

FIG. 2.

early in their existence, thus proving their cell nature. He also states that in an adult these can be vaguely seen. In no eye did I see an indication of nuclei in the segments of the crystalline body.

Cross-sections show the segments to be arranged in no regular manner. In a complete series of cross-sections one ommatidium was followed, and camera drawings at the high and low level were made of each section.

It was thus possible to trace each segment and find the number of segments and their relative position. In most ommatidia the number counted was ten to eleven, but in one ommatidium I was able to trace twelve. It must be understood that in a single section no more than six to eight appear. This can be seen by referring to Fig. 2.

Summary.

The species described by Grenacher is Scutigera (Cermatia araneoidea); by Adensamer, Scutigera coleoptrata; by myself, Cermatia forceps.

The latter is the only American Scutigera.

The differences in the accounts are probably to be explained in part by the fact that the individuals studied were of different species.

(1) The number of ommatidia in each eye of Scutigera forceps is about two hundred. In Cermatia araneoidea (Grenacher) the number is given as one hundred.

(2) The crystalline body was found to be made up of ten to twelve segments, instead of six to nine. No nuclei were observed in these segments.

Each ommatidium is made up of the following cells:

(3) Elongated pigment cells surrounding each ommatidium, sixteen to eighteen in number (p, Fig. 1 *A*).

(4) An outer tier of pigment cells, embracing the distal portion of the crystalline cone, ten to twelve in number (t_1, Fig. 1 *A*).

(5) A middle tier of cells of ten to twelve (t_2, Fig. 1 *A*).

(6) An inner tier of cells situated at the proximal end of the

ommatidium, touching with their proximal ends the basal membrane. In the inner tier there are from three to four cells (t_3, Fig. 1 *A*).

The cells of (5) and (6) secrete, upon their inner surfaces, rhabdoms, and from the outer side send out a process constituting the nerve fibers.

These nerve fibers were mentioned by Adensamer, but his figures failed to show the direct connection between the fibers and the cells of the middle and inner tiers.

In macerated preparations I have been able to show this beyond doubt (Fig. 1 *C*, *D*), and in the ommatidium, before it has been separated into its component parts, have observed the passage of these fibers through the basal membrane.

The expanded proximal portions of the elongated pigment cells (Fig. 1 *B*, *p*) form the layer of pigment spoken of by Adensamer, as found on the basal membrane.

(7) Supplementary cells, four in number, are found at the same level as the cells of the inner tier. They are entirely different, and thus easily distinguished from the cells of the inner tier (*a*, Fig. 1 *D*).

(8) As shown in Fig. 2 *A* certain large nuclei were found in cross-sections at the distal part of the ommatidium. They are found only in the space between three ommatidia. They were not observed, nor the cells to which they belong, in maceration preparations.

Bryn Mawr College, April, 1900.

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UNUSUAL MODES OF BREEDING AND DEVELOPMENT AMONG ANURA.

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IT has been my good fortune to obtain from Jamaica a complete series of stages of the development of *Hylodes martinicensis*. The eggs of this frog are deposited on land, and the entire development takes place within the egg-membranes. The stages that correspond to the tadpole of our common frogs are passed through inside the egg-membrane and a fully formed frog emerges at the time of hatching. In connection with the study of the development of Hylodes it has been necessary to collect the descriptions that have been given of exceptional modes of development in the Anura. These accounts are often very fragmentary and widely scattered, so that no small labor has been necessary to bring together the literature of the subject. In the hope that a consecutive and full account of what is known may be of service to others, the following pages have been prepared.

In the groups of fishes and Amphibia the eggs are generally laid in large quantities and are left unprotected, but there are other exceptional modes of propagation in species of

unrelated genera and families. In these cases protection of some sort is generally afforded to the young, or else they have become adapted to an unusual environment. As a result, modifications in the young have been produced, as well as changes in the instincts and even in the anatomy of the parents. Examples among fish are the various cases of nest builders among teleosts ; the cases of Bagrus, of the siluroid Chromis, and of other fish that carry the young in the mouth for a longer or shorter period ; the case of *Aspredo lævis*, in which the female carries the eggs and young attached to pedicels on the abdomen ; the cases where the male fish is provided with a temporary or permanent pouch for the young, and the cases of partially or entirely viviparous forms among teleosts and selachians. Examples among Amphibia are the viviparous salamander ; Plethodon and Autodax, which lay their eggs on land ; Desmognathus, Amphiuma, and the Cœcilia, where the eggs are laid in damp places and are surrounded by the parent's body ; and a number of Anura, which are the subject of the present paper. For the sake of comparison with what is to follow, the salient points in the development of the common frog may be briefly stated. The eggs are laid in large numbers in water, where the larvæ develop. The newly hatched tadpole adheres at first to a fixed object by means of an adhesive gland, but after a few days it becomes free-swimming. It possesses at first external gills; these are replaced by internal gills, covered by the characteristic operculum ; the internal gills are in turn gradually replaced by lungs ; in the mean time the hind legs and later the fore legs develop ; the tail is absorbed, the shape and position of the mouth are changed, the larval "mouth-parts" are cast off, and the characteristically coiled intestine is shortened. The metamorphosis transforms the aquatic larva into the adult air-breathing form.

This general account is true for all the common frogs and toads of North America and Europe, with the exception of Alytes. The Anura of other regions, especially in the Tropics and the Southern Hemisphere, far from having uniform breeding habits and one mode of development, vary

in many ways. For the sake of completeness, a number of forms will be mentioned that are often referred to in connection with those that have an unusual mode of development; they are forms whose embryology has been but little, if at all, investigated, but whose development takes place under somewhat unusual conditions, so that the embryology is a question of interest.[1]

The eggs of the Japanese frog, *Rhacophorus schlegelii*, are laid from the middle of April to the middle of May in excavations prepared by the female in the muddy banks of paddy-fields, ponds, lakes, etc., 10 to 15 cm. above the water line. As the eggs are laid, they are fertilized, and the gelatinous substance about them is aërated by a kneading motion of the hind legs of the female. Both adults usually escape from the nest by breaking a hole in the side of the bank exposed to the water. The frothy egg-mass hardens on the outside, and within it the tadpoles hatch. The substance about the eggs gradually collapses, and in time flows into the water, carrying with it the tadpoles. The eggs are about 1 mm. in diameter, and devoid of pigment until the tadpole stage, when pigment begins to show in the pectoral region. The segmentation is said to be unequal and holoblastic, though more nearly meroblastic than in any other amphibian egg.[2] The embryo is described as resembling a ganoid embryo, the head being flat upon the yolk, and the body wedged into the large yolk-mass.

It is interesting to note that eggs taken from the nest in the mud invariably die if put into water.

Eggs that are said also to be those of Rhacophorus have been found in various localities in Japan, in a frothy, gelatinous substance on the leaves of trees overhanging the water. How the eggs are deposited is not explained.

Two other frogs, from different parts of the world, leave their spawn on trees, in nests formed of leaves stuck together. One of these, the West-African *Chiromantis guineensis*, is found

[1] For an index to the forms discussed, with summary of literature referring to each, see Appendix, p. 714. References to reviews are given after special articles.

[2] The segmentation of several other anuran eggs that contain much yolk has not as yet been described.

in Cameruns, Victoria; the other, *Phyllomedusa iheringii*, in the province of Rio Grande, Brazil. In both cases hatched tadpoles have been seen, moving with considerable freedom, in the frothy mass in which the eggs are laid. The nest of the Phyllomedusa has an inferior opening over the water. It is believed that the tadpoles of Chiromantis are washed off the leaves by rain. Tadpoles, hatched in confinement, at first swam in the gelatinous substance which liquefied about them, and being transferred to water, they lived and developed. The account of Chiromantis opens with an exclamation on the remarkable character of the metamorphosis of the frog, and yet the tadpoles are described as having a "rudder-tail, a clump of gills, etc., just like an ordinary tadpole," and they "developed (in water) in the usual way."

The little *Phyllomedusa hypochondrialis*, in Paraguay, selects for the deposition of its eggs a single leaf on a plant near a pool, or over it, not more than two feet above the water. The female carries the male, until a suitable leaf is found; then both frogs hold the tip of the leaf together with their hind legs, and in the funnel thus formed the eggs are deposited, the frogs moving up the leaf as the bottom is filled. One pair of frogs may make two nests with about 100 eggs in each. The eggs contain much yolk and measure 2 mm. in diameter. If put into water, the eggs die, but develop if they are simply kept moist. The tadpoles hatch and escape from the leaf into the water after six days; if the nest has not been directly over the pool, the tadpoles may move (assisted by a jumping motion) several inches along the ground during a shower.

Segmentation is holoblastic, but not very regular; the blastopore closes and a new anus is formed. The embryo at first lies flat upon the yolk, but before the yolk is absorbed the embryo is modeled high up upon it, and the head and tail are entirely separate from it. The yolk is covered by a plexus of vitelline veins.

Three visceral pouches, the 1st, 2d, and 3d branchial, are formed, and external gills appear first on the 1st branchial arch and later on the 2d. The first pair grow rapidly and soon become very long, branched filaments; the second pair

never develop to the same extent. No adhesive gland is formed. When the external gills are developed the tail has grown to a large size and has so ample a blood supply from the dorsal aorta and cutaneous veins that it is supposed to be an important organ of respiration. After hatching, the tip of the tail often vibrates rapidly, perhaps to insure the flow of a constant stream of water over the tail.

Before hatching, the operculum grows back from the hyoid arch, and has a median spiraculum; the external gills are rapidly absorbed, and internal gills are developed. The stomodæum breaks through, at the last stage in the egg, and the tadpole hatches as a free-swimming, transparent larva, with prominent eyes, and with no longer any trace of yolk. The lungs are present. On the day after hatching, pigment appears in the larva.

Five weeks later the hind legs begin to develop, and the tail is absorbed very rapidly, until only a little of it remains. After the legs are formed the frog leaves the water, and the final absorption of the tail takes place on land.[1]

The eggs of *Hyla nebulosa*, found in Rio de Janeiro, hatch in a frothy mass in the sheath of withered banana leaves, but not near a pool. Unlike the tadpoles of Chiromantis, which probably reach the water in the natural course of events, the tadpoles of *Hyla nebulosa* invariably die if they are transferred to water.

The tadpoles of *Cystignathus* (*Paludicola*) *gracilis* in Brazil, and of a frog believed to be *Rhacophorus eques* in Ceylon, are said to undergo part, at least, of their development out of water. The eggs have been found in frothy masses on land, those of Cystignathus usually in the grass in the neighborhood of pools. The adult Cystignathus, and on one occasion two young, were found under decaying trunks of trees, near dried ponds; the larger of the two young possessed the vestige of a tail. The naturalist d'Orbigny found the same species in the Argentine Republic, where it occurs under pieces of wood, near the borders of lakes that are common in the sandy soil. The tadpoles of Rhacophorus were observed superficially and are said to resemble those of the ordinary frog.

[1] For further details, see Budgett ('99).

The adult of the Brazilian frog, *Cystignathus mystaceus*, in the province of Rio Grande do Sul, never goes to water, even to spawn. In the breeding season it prepares a hole under a stone or decayed wood, near the edge of a pool, but above the water line; here the eggs are deposited in comparatively small numbers. The frothy substance about them probably serves as food for the tadpoles, since it diminishes in quantity as they grow. In a dry season the tadpoles may attain great size in the nest, but ordinarily they are washed into the pool, when, after a rain, it overflows. They probably do not go through the entire metamorphosis in the nest, however long they may be detained in it, for tadpoles that had grown there to a considerable size still possessed tails. During development in the nest, external gills were observed; the tail was thought to be not so powerful as that of *Rana esculenta*, and is at first light in color, like the egg, but later becomes pigmented dorsally. Another Brazilian nest builder is *Hyla faber*, which is common in Rio de Janeiro, especially in the mountain regions. The female constructs for the eggs a shallow vessel of mud, about four inches high and a foot in diameter. The nest is carefully built on the bottom of a pool, with its edge projecting above the water. Similar nests have been found in Rio Grande do Sul,[1] and there it has been observed that, if the pool dries, the tadpoles in their little lagoons perish, while the young of *Cystignathus mystaceus* survive, huddled together in the frothy substance in their nest in the bank. The eggs of *Cystignathus typhonius*, a frog common in Porto Rico, have been found under conditions similar to those described in the case of *C. mystaceus*. The tadpoles swam when put into water. The appearance of the hind legs and of the fore legs and the disappearance of the tail were observed.

In Ceylon, a green frothy spawn, about the size of a crow's egg, has been found sticking to the walls of cisterns, to perpendicular rocks of quarries over water, and to the damp

[1] It was formerly supposed by the observer in Rio Grande do Sul that such nests were constructed by *Cystignathus ocellatus*, but there is good reason to suppose that *Hyla faber* is the artisan. In Rio de Janeiro this frog has been observed in the very act of building, which takes place at night.

trunks of trees. After some discussion it has been decided that the spawn is that of *Polypedates maculatus*. A very similar account is given of the blue spawn of *Rana temporalis*, also found in Ceylon. The two descriptions agree so closely that one is led to suspect that the two cases may be one and the same.

The tadpoles of a Brazilian frog, *Hyla (Ololygon) abbreviata*, have been found in cracks of rocks. The abdomen of the tadpole is flat and serves as a sucker, so that if the perpendicular rock is slightly moist, the tadpole can move rapidly over it without legs. The tail is round, with a fin only at the end; on the ventral side the fin is anteriorly converted into a sole, which probably aids the tadpole in adhering to the rock. The mouth is large and the lips are unusually developed. It is said that the frogs, for a time after metamorphosis, are the color of the rocks. Nothing is recorded as to where the eggs are laid, or how they develop.

The breeding habits of *Pipa americana*,[1] commonly known as the "Surinam toad," have been a subject of observation, still more of discussion, from time to time for more than a century. It has been found in British as well as in Dutch Guiana. The frogs are said to be essentially aquatic, and rarely to leave the water. The eggs are laid in the dry season, when the temperature is exceedingly high. The earlier accounts of spawning are probably based on the observations made by Fermin in Surinam, and published in 1765. He states that the eggs are laid on the sand and are placed by the male on the back of the female; a few minutes after fertilization the female returns with them to the water and swims off. The spawning has recently been described in a different way, as observed on one occasion in the Zoölogical Gardens of London. The animals remained in the water, the oviduct was protruded into a bladder-like pouch, turned up over the back; the male clasped the female, and pushed the eggs out of the bladder, leaving them evenly distributed over the back of the female. It is supposed that the eggs are fertilized in the ovipositor.

[1] A few embryos of Pipa have been in the possession of the Warren Anatomical Museum of the Harvard Medical School.

The dorsal skin of the female thickens about the eggs, until each is enclosed in a dermal "cell" or sac, which is finally covered by an "operculum." Leydig ('96) regards the cells as modified glands, and, in corroboration of his view, states that other dermal glands are not found where the cells are formed; he regards the cap of the cell as a secretion of the gland, while formerly it was thought to be a hardened portion of the gelatinous substance around the egg. Whatever the origin of the cells, they are temporary structures which form in response to the stimulus of the eggs; the number of eggs carried by one frog has been variously estimated, and may be more than one hundred.

The eggs develop entirely within the dermal cells, and Fermin has observed that they remain about eighty-two days on the back of the parent. I have found no direct statement as to whether the time of hatching is coincident with the time of leaving the dermal sac, except in Bronn's Thierreich. There it is said that "the hatched young find shelter and nourishment on the parent's back, until after completed metamorphosis," implying that the eggs are hatched some time before the young escape.

The only description of development, based on the observation of many embryos, is one by Jeffries Wyman ('54), giving the external characters of three stages.

The limbs develop early; even in the youngest stage, where there were three pairs of external gills, the fore and hind legs were present, in the condition of knobs at the sides of the embryo, the posterior said to be unconnected with the trunk. There were at this stage vitelline vessels, supplying the large yolk-mass. The head was broad and flat, with conspicuous cerebral vesicles and prominent pigmented eyes; the spinal cord was closed.

Later (in the second stage), when the external gills had disappeared, fringed branchial arches were seen by removing the dermal folds which concealed them; the opercular folds opened by a small branchial fissure on each side of the neck, and judging from the figures, did not cover the anterior legs. Rudiments of feet were present on all the extremities. It is

said that the whole yolk-mass was invested with a "tunic" and converted into a spiral intestine, returning to the trunk through the middle of the coil! The nostrils in this stage were seen as round terminal depressions.

In the last stage toes were formed; the skin was furnished with papillæ and protuberances; the intestine had increased in length and was still coiled; the mouth, as in the preceding stages, was placed under the head, at a little distance from the anterior end. Another account states that the horny jaws are not developed. The tail in all embryonic stages was provided with the usual muscles as if for swimming, and was folded against the side of the egg. Wyman shows that, after the supply of gelatinous substance around the egg has been exhausted, the tadpole still continues to increase in size, and he concludes that it must therefore grow at the expense of material from the mother, probably by a secretion of the wall of the cell. In summing up the development of Pipa, Wyman notes that the embryo goes through the usual metamorphosis, having internal and external gills and lungs, also a tail adapted to swimming, although the gills and tail are never used as in the frog. The external branchiæ are lost at a very early period, and the tail is absorbed before the animal escapes from the back of the parent.

The folds of skin covering the internal gills differ from the operculum of the frog in that they open on both sides, and the anterior legs are not covered by them. These relations are similar to those in the larva of Dactylethra, the other representative of the primitive suborder of Aglossa. The presence of external gills has been doubted at various times, but the balance of evidence and the last word on the subject seem to show that they exist for a short period.

An early writer, according to subsequent quotations, affirmed that the tail is devoid of pigment, and believed that it is an embryonic breathing organ. This idea was entertained at a time when the presence of gills was not known; it is not in accordance with Wyman's account, nor with Spallanzani's (1785), who concluded, after a careful examination, that the tail does not differ from that of the ordinary tadpole. Furthermore,

Bronn and Balfour state that the tail is absorbed before hatching, and Bronn has implied, as already mentioned, that the hatched young remain for a time on the parent's back. If these statements be true, and if it were also true that the tail assumes the function of respiration, then the animal would be deprived of the embryonic breathing organ some time before it escapes from the cell on the parent's back.

Alytes obstetricans [1] is unique among European frogs in not laying its eggs in water. It occurs in various parts of Europe, such as the Rhine region in Germany, the neighborhood of Marbourg, Paris, and Liège. It has been said that it lives only in regions of hilly ground, where there are clear springs ; but Cope ('85) records that it is common in dry places near Berlin. The frog digs skillfully, and remains underground by day, but at night it is active in the search for food.

The eggs are laid from March to June (according to locality), in a double string of gelatinous substance. The male loops the eggs about its hind legs in the form of a figure 8, and thus carries them, for about three weeks, when the young have reached a stage of development far beyond that of the typical tadpole at the time of hatching. At the end of the period the male takes the eggs to water, and there the tadpoles leave the egg-membrane, and at once swim about actively. They are said to winter in the tadpole stage, although Claus ('66) has affirmed that the metamorphosis is completed in a month. When the tadpole leaves the egg, the external gills are already reduced, and the internal gills are fully developed ; the opercular opening is median ; the tail is ready for swimming ; the lungs are developed ; the horny jaws, teeth, and fringed lips are formed (these are said to appear later than in the ordinary frog) ; the intestine is coiled, and the liver is present ; the head kidney is in process of degeneration, and the mesonephros partially developed ; the legs are not formed. It has been suggested that the lungs are used before hatching, because air bubbles are given off as soon as the tadpoles reach the water.

[1] A complete account of Vogt's ('42) article is not given here. Gasser ('82) also describes details not mentioned here, especially in regard to the blastopore, the germ layers, and the development of the pronephros and kidneys.

The egg contains an unusual amount of yolk, and is not pigmented; the segmentation was formerly thought to be meroblastic, but it has recently been described as similar to that of Bombinator. The embryo is said to be ciliated even on the head. Certain peculiarities in the development of the alimentary canal have been recorded. The cavity is at first wide, and in the foregut and hindgut it remains in this condition, the anterior end having the widest lumen; in the middle region the ventral yolk-mass is raised in a ridge, toward the dorsal wall, so that the midgut is, for a time, nearly or entirely barricaded. An early description seems to indicate that the œsophagus is at one time closed by yolk-cells, as in the frog. The liver is said to develop independently, and shortly to acquire an opening into the alimentary canal. It is also said that the vitelline veins are at first not connected with the liver, but pass into it later as the portal vein. The lungs arise as solid masses of cells in the œsophageal region, and subsequently become hollow. An adhesive gland is not mentioned.

Before the gills develop, four gill-slits break through; the external gills are then represented by a single pair, on the third visceral arch; they grow to an unusual length during the life in the egg, and are branched eight or ten times. These delicate plumes are very highly vascular and therefore bright red, and are easily seen through the egg-membrane.

When the tadpoles are about ready to leave the egg, the hatching may be hastened or retarded within the limits of two or three days, by bringing the eggs into water a little before or after the normal time. Tadpoles that were put into water long before the usual time, for example when the external gills were fully developed, fruitlessly turned within the egg-membrane, and finally died.[1] On the other hand, Agassiz tried without success the converse experiment of raising the newly hatched young in air.

Some interesting facts have been discovered in connection

[1] The experiment of first cutting away the egg-membrane and then leaving the tadpoles in water was apparently not tried. Compare experiments on the embryos of the viviparous *Salamandra atra*. Those taken from the uterus, if tended with great care, cast off their gills in water and develop a new set. Chauvin ('**77**).

with the Venezuelan frog *Nototrema oviferum* (*Notodelphys ovipara*, *Opisthodelphys oviferum*), found at Puerto Cabello. The female is provided with a large dorsal pouch, which opens to the outside at its posterior end. It has been regarded as an invagination of the skin, and unlike the dorsal cells of Pipa it is a permanent organ. Weinland ('54) has described fifteen embryos that were contained in the pouch of one specimen, and had all reached the same point in development. They measured 18 mm. from head to tail, and were folded over a large yolk-mass, that formed about seven-eighths of the egg. The embryos were dark with pigment, but over the yolk there were only scattered pigment cells. The rudiments of body muscles are described as present beneath the skin about the yolk. The head was well defined and of the shape peculiar to the adult, with prominent eyes and semicircular nasal openings. The mouth was ventral near the anterior edge of the head, and measured little less than one-half the width of the head. It is said in the course of the description that the horny jaws were not developed, although at another place in the same account the embryo is paralleled, in respect to the mouth, with the newly hatched Alytes, where the tadpole mouth-parts are present.

In the embryo of Nototrema no adhesive gland was found, and the tongue was not developed. The fore legs measured about 2 mm. and were concealed beneath the operculum; the hind legs were twice as long, the toes were forming. The tail measured 5 mm. in length and 2 mm. in width, and with the hind legs was laid against the side of the egg. The heart was three-chambered, and the aortic arches separated not far from it. The dorsal aorta, posterior vena cava, and portal vein from the gut to the liver were large vessels. The lungs were well developed. The three-lobed liver, the gall bladder, and large pancreas were present, the pancreas surrounded by a coil of gut, leading to the stomach portion. The foregut and also the hindgut were thick-walled and appeared white, while the gut between them was formed of coils packed with yellow yolk. The pronephros had entirely disappeared, and the fat bodies and the kidneys were present.

The most remarkable structures were the gills. Three visceral arches were present, and according to one statement three gill-slits (three slits appear in the figures, but in another part of the description it is said that the first and third arches have each only one free edge — a condition that would admit of only two slits). The gills consisted of a pair of very thin bell-shaped membranes, each gill connected with the gill arches by two filaments, one ending on the first and one on the second arch. This peculiar arrangement led Weinland to the conclusion that the bells represent two gills fused together. He regarded them, in function at least, as external gills. On the free edge of the third arch was found the rudiment of what was supposed to be an internal gill, which suggested that the bell-gills are temporary larval organs. The bell-gills were veined by a capillary network, and the vessels were filled with blood, so that the gills were probably already functioning, although pressed close to the embryo in the egg; it was only after floating them out in water that their windflower shape and large size were discovered. The gill measured across, when expanded, not less than three-quarters of the diameter of the egg. The gill filaments were provided with striped muscle fibres; these muscles, Weinland argued, could not be of use in the egg, but the gills may be retained a short time after hatching, when the muscles might function in water, whether the tadpole remained within the pouch or escaped.

Weinland knew nothing of the subsequent history of the tadpoles, as to when they hatch and whether they ever live in water, either free or within the pouch; Boulenger makes some unqualified statements in regard to these questions.

He has noted the occurrence of eggs in the dorsal pouch of the female of *Nototrema fissipes;* he observed a single specimen, in which there were sixteen ova, measuring each 10 mm. in diameter. "From the size and small number of the ova," he writes, "it may be safely predicted that the young undergo the whole of the metamorphosis, within the pouch, as in *Nototrema oviferum*, which is the nearest ally of *Nototrema fissipes.*" Of two species of Nototrema found in Ecuador and Peru, also provided with pouches, Boulenger says in an earlier work ('82),

"In *Nototrema testitudineum*, as in *Nototrema oviferum*, the young undergo their complete metamorphosis in the maternal pouch; while in *Nototrema marsupiatum* they leave the pouch in the tadpole state." The tadpoles of *Nototrema marsupiatum*, and those of another species, *Nototrema plumbeum*, are set free in water, according to Werner ('98).

Yet another frog of the tropical Andes, *Amphignathodon güntheri*, is said to have a dorsal pouch, which is probably a receptacle for eggs.

The female of a small Venezuelan species, *Nototrema pygmæum*, bears from four to seven young in a dorsal pouch, which splits open when the development of the tadpoles is complete. The pressure of the active young starts the splitting of the pouch at the slit-shaped posterior opening, and the whole pouch is laid open in the middle line. The pocket is perhaps formed from lateral folds after the eggs are placed on the back, and is possibly not closed again after the young escape, but is formed anew at the next breeding season; or the pouch is perhaps never formed a second time.

Observations on a number of tadpoles of *Rhinoderma darwinii* have disclosed some of the facts of the development of this interesting frog. It was first discovered on the voyage of the *Beagle*, in the shady forest of the province of Valdiera in Chile. It was formerly supposed to be oviparous, but, in reality, the tadpoles develop in an enormously expanded gular pouch, which covers nearly the entire ventral side of the male frog.

One account tells of fifty embryos in the pouches of five adults, and a much later account describes certain features of eleven tadpoles of one pouch. The tadpoles from one adult are not equally developed.

The youngest tadpoles described were thought to be just hatched. They measured 4 mm. to the tail; the alimentary canal was not differentiated, but contained much yolk. In all later specimens the alimentary canal was short, coiled, and of a yellow color, due to the presence of yolk. No external gills were observed, nor horny mouth-parts. Internal gills are not mentioned, although the anterior legs in young stages are concealed beneath a fold of skin (suggesting the operculum).

The hind legs develop before the fore legs and in late stages the feet are webbed. The tail is weak and small, and in the youngest embryos lies along the middle line of the egg-sphere. The oldest tadpoles had lost the tail, but at a period before its absorption both fore and hind legs were present and free.

The adults of *Hyla gældii* have been found in Rio de Janeiro in water, contained in the central cup of certain Bromeliaceæ, and also on dry bamboo near Bromelia. The dorsal skin of the female is slightly raised around the edge of the back, forming a shallow cup, where the eggs are carried. In one case the dorsal vessel contained twenty-six pale eggs. Judging from the figures, the embryos, in external appearance, resemble to a remarkable degree those of *Hylodes martinicensis*. They are unpigmented and are bent around a large yolk-mass, 4 mm. in diameter. The head, which is large and flat, and the eyes may be seen through the egg-membrane. No traces of gills were discovered. The accounts vary as to the condition of the embryo when hatched. According to Goeldi ('95), frogs that hatched in confinement possessed both pairs of legs and small tails; the young jumped about actively and preferred not to stay in water. No details are recorded, but it is implied that the development is abridged and direct. Werner ('98) affirms that the tadpoles are ready to swim when hatched, and that the mother finds water to deposit them in. According to Fritz Müller's description in a letter to Darwin, the tadpole which he observed did not possess fore legs until two weeks after hatching; he saw no external branchiæ or opening that might lead to internal branchiæ.

Another mode of carrying eggs by the female frog is seen in the case of *Rhacophorus reticulatus* (*Polypedates reticulatus*). A single specimen, which was captured at a high elevation in Ceylon, carried twenty-one ova adhering firmly to the abdomen. It is uncertain whether the frog was caught in water or on land, and nothing has been noted as to the development.

An interesting adaptation in a dry climate is recorded of a species of *Spea* (*hammondii?*) in western North America. The eggs are deposited in rain pools, which collect in the dry arroyas and in the low lands. The tadpoles acquire their legs

very early, and are soon able to escape from the muddy fluid; thus, by accelerated metamorphosis, the chances of succumbing because of drought are lessened.

Lythodites latrans, which lives in fissures of limestone precipices of western Texas, is also supposed to deposit its eggs in pools of rain water.

The eggs of *Phryniscus nigricans*, in Paraguay, are laid singly, in temporary pools in grassy ground. "The eggs and larvæ do not seem to differ in any great degree from those of Rana. There is, however, a very large yolk plug, which remains evident after the closure of the neural groove." The development is very rapid, and, within twenty-four hours after segmentation begins, the tadpoles are hatched and wriggling about. They are probably washed into deeper pools.

Another case of rapid development is that of *Paludicola fuscomaculata*, also found in Paraguay. It spawns in January in shallow pools, and the eggs float on the surface. They measure 1 mm., are unpigmented, and have very little yolk. The tadpoles hatch within eighteen to twenty-four hours after segmentation begins, and hang in the water from the floating froth. "Many of the processes of development are blurred and, as it were, hurried over. The external gills never reach a high state of development."

Eggs which are thought to develop like those of Paludicola are said by the natives of Paraguay to belong to the frog called by them "Po it," more scientifically *Eugystoma ovale*. Eggs and larvæ are found in froth in holes in the ground, beneath fallen tree trunks. The nests "were a most ingenious contrivance for collecting water and keeping the eggs and larvæ at least moist between the storms of the wet season. They were always found within the forest belts which lay on the highest ground." The larvæ "would exist for a very long time in a small quantity of water without increasing in size, but when removed to a tank they grew enormously and very soon left the water."

A curious indifference to water has been observed in the case of *Spea bombifrons*, near Market Lake, in Idaho. The tadpoles, still "in the free-swimming condition," but entirely

air-breathers, were seen quite out of reach of the water, in small spaces which they had cleared in the ground. The legs were full-grown, the tail undiminished, the jaws toothless and cartilaginous and "some quite larval in form."

Two species of Dendrobates resort to a device by which it is supposed that the young are transferred from one pool to another. The accounts are sometimes referred to as if they related to a single species. The species closely resemble each other, except in size; the larger, *Dendrobates trivittatus*,[1] is found in Dutch Guiana, the smaller, *Dendrobates braccatus*,[2] occurs in western Brazil. These frogs and *Phyllobates trinitatus* from Trinidad and Venezuela have the habit of carrying their tadpoles attached to the back, whether by suckers or by a viscid secretion from the parent, or by both, has not been determined. It has been observed in the case of *D. trivittatus* that the frog spawns in water, and that the free-swimming tadpoles attach themselves to the parent. They adhere firmly, since they were not brushed off when the parent was hotly pursued and crawled rapidly through the grass; the frog on this occasion was found far from water, and emerged from the grass and bushes freshly wet with rain.

D. braccatus is found on a table-land where, even in the wet season, the water may dry off in two or three days.

The tadpoles of both species possess the usual larval organs, including the horny mouth-parts. The intestine of the tadpole of *D. trivittatus* is said to be shorter and less coiled than in the ordinary tadpole, and to contain yolk.

In the case of Phyllobates it is the male frog that transports the young, but the sex of the frog is not recorded in either case of Dendrobates. The tadpoles of Phyllobates are ranoid, and the tail is about twice as long as the body; the anus is dextral, and the spiraculum sinistral.

[1] *Dendrobates trivittatus* has been wrongly called by one observer (Wyman, '59) *Hylodes lineatus*.

[2] Smith, in describing *Dendrobates braccatus*, reports that the natives of Brazil brought him from the Santarem forest a similar, though larger, frog and affirmed that, like *Dendrobates braccatus*, it transports its young. This may have been *Dendrobates trivittatus*, which is described by Wyman and others, since the difference between the two species is one of size.

A case which resembles Phyllobates and the like, superficially at least, has been discovered by Brauer ('98) in the Seychelles. *Arthroleptis seychellensis* inhabits a forest about 500 feet above the sea, where the streams are swift and there is no still water. The frogs are found in decayed trunks and among damp leaves on the ground, and on one occasion, late in August, a number of eggs were found under leaves from which the adult had been frightened away. The eggs were not included in a common gelatinous covering; they hatched in a glass, and the tadpoles immediately stuck to the glass by their abdomens. Tadpoles of about the same stage of development were also found adhering to the back and sides of an adult. The tadpoles had prominent eyes, mouth well under the anterior end of the head, short hind legs, fore legs concealed beneath an operculum, rudder tails more than twice as long as the body, and a large amount of yolk bulging out on either side of the body. No opening was found to the operculum, and no internal gills, nor were the rudiments of lungs discovered. The outer layer of epithelial cells on the abdomen was columnar, making the epithelium appreciably thicker there than elsewhere; gland cells were wanting on the abdomen only, though a slimy secretion covered the surface. An adult carrying older tadpoles was found at another time. Gland cells were present on the abdomens of these tadpoles; the anterior legs had come through the skin, the toes were found on all the feet, the length of the tail was not reduced in proportion to the body, and much yolk was still present. Rudiments of lungs were found, and the entodermic epithelium had differentiated from the yolk.

Brauer concludes that the eggs are covered with leaves by the parent (probably the male), that the young, when hatched, place themselves, with the assistance of their tails, on the parent's back, where they adhere partly by suction, partly by aid of the roughness of the parent's skin, and partly by a secretion, largely from the adult. Finally, the tadpoles are not attached to the parent for transportation to water, but undergo a large part of their development on its back.

Hylodes martinicensis has been described as the "coqui"

found on several of the islands of the West Indies. Superficial descriptions of the eggs are recorded by two writers (Bello, '71; Peters, '76). The eggs were found on damp leaves of plants, and near them was the female, apparently on guard. The frogs hatched in the adult form after fourteen days. The embryo is described as wrapped about the yolk, like a mammal. On the seventh or eighth day both pairs of legs were present, and the tail, eyes, and pulsating blood vessels were observed. On the twelfth the characteristic toes and suckers had appeared. The tail was turned downward, with the broad surface pressed against either the right or left side of the embryo. There was no trace of gills or gill-slits, but the tail was so fully supplied with blood that it was regarded, without doubt, as a breathing organ. On the fourteenth day newly hatched embryos were 5 mm. in length, and the tail, which measured about 1.8 mm., was absorbed during the course of the day.

In 1873 there appeared in several magazines various accounts, by Bavay, of a frog which he called *Hylodes martinicensis*. The accounts differ in some respects from those of the other observers of Hylodes, but are not altogether consistent among themselves, so that Bavay's observations were probably not very accurate. He affirms that in one locality, at least, spawning does not take place during the dry season, or even after lack of rain in the wet season; the eggs are always found in damp places, under stones or with decaying leaves. Instead of fourteen days, he allows only ten or eleven for hatching, and states that the frog goes through the usual metamorphosis within the egg. The eyes, legs, tail, and feet he describes at a period earlier than that in which they were found by the other observers, but in regard to the tail his own accounts vary; he stated at first that it disappeared two days before hatching, but afterwards announced in a published letter that he found a vestige of a tail in tadpoles just hatched, when they were hardened in glycerine. The vitellus, according to his observations, was at first connected with the embryo by a cord, for the embryo on the fourth day moved independently of the yolk-mass, although at the same time both

rotated together in the egg. On the sixth day the yolk was covered with a vascular network, and on the ninth it had been incorporated in the body. On the fourth day, when the embryo was turning actively, the heart was beating, and on either side of it there was a trace of a gill; a vein and artery were seen running parallel in the gill. In the course of the next few days the gills had first increased and become blood-red, then diminished, and on the seventh day only a red spot remained, which disappeared on the ninth. Bavay's observations were made in Guadeloupe, and it is possible that on this island there is a frog differing from *Hylodes martinicensis* in the points indicated by Bavay's description.

A study of two stages of the excretory system of Hylodes led Selenka ('82) to the opinion that the embryonic and permanent kidneys develop earlier than in the frog, producing a condition which approaches that of reptiles.

It would be interesting to know something of the development of *Hylella platycephala*, a frog which occurs in southern Mexico. It is said to lay its eggs in the axils of the leaves of Tillandsia, where the frog "undergoes its metamorphosis, high above the ground."[1]

Whether the entire development occurs within the egg-membrane, and whether water continually remains on the plant is not stated.

A short note on a frog, supposed to be *Hylodes lineatus*, records some evidence that its embryos undergo their entire development within the egg-membrane. The eggs have been found in Peru under grass far from water, and the embryo not yet hatched possessed no trace of a tail, but well-developed feet provided with suckers.

The young of *Rana opisthodon* hatch in the adult condition. Eggs were collected in crevices of rocks close to a stream on a peak of one of the Solomon Islands. The eggs measured from 6 to 10 mm. in diameter. In the younger stages the

[1] Andrews ('92) reports that the eggs of a tree frog in Jamaica have been found in the water at the bases of the leaves of epiphytic bromelias. They were observed in early stages of development, at the end of May, in the region of Mandeville.

embryo was folded around a large yolk-mass in the same way as *Hylodes martinicensis*. The tail was entirely wanting in the more advanced specimens. No traces of gills were found, but on each side of the body were several transverse folds of skin that were thought to be respiratory organs. The legs were well developed; the anterior were shorter than the posterior. The tip of the upper lip was furnished with a small conical protuberance, which in one case projected through the envelope of the egg, suggesting that its function is to perforate the egg-membrane.

The South African frog *Dactylethra capensis*, like *Pipa americana*, the other representative of the Aglossa, lives entirely in water; the tadpoles of the two forms agree in having the fore limbs free from the operculum and in having two spiraculæ.

Leslie ('90) writes of Dactylethra (called by him Xenopus): "Its habits are essentially aquatic, the animal never leaving the water except in search of places where food or shelter is better supplied. Unlike other frogs, it feeds in the water, on insects, small fishes, or even young and larvæ of its own kind, and is apparently unable to feed out of that element. The mode of eating is by forcing the prey into the mouth by means of the hands, which act as a pair of claspers; the deglutition always takes place under water. Locomotion on land is by difficult and awkward crawling and leaping. But Xenopus is a most admirable swimmer, and remarkable for the manner in which it remains poised for a long time immediately under the surface of the water, with the nostrils only exposed. The whole structure of the animal denotes its thoroughly aquatic habits — the broadly webbed toes, the smooth, slimy skin with its symmetrically disposed muciferous tubules; there are no eyelids proper, but merely the transparent nictitating membrane, moving up and down; and the nostrils have a disk-like internal valve. When at rest, Xenopus never assumes a sitting posture like other frogs and toads, and the back never appears humped."

The eggs (measuring about one-sixteenth inch in diameter) are laid singly and attached to water-plants or stones, each

surrounded by a mucilaginous covering. They are at first very slightly pigmented. Tadpoles in the Zoölogical Gardens in London hatched in about two days; the head of the newly hatched tadpole is broad and flat; the fin is developed on the tail to the posterior end, and there is no chimæroid lash as Parker ('77), and subsequently the text-books, have figured; the intestine is coiled as usual. The tadpoles in confinement had access to vegetable food, but subsisted entirely on an animal diet. A single ventral adhesive gland persists for a long time and at an early period occupies the whole ventral surface of the head. The buccal cavity is open to the exterior the day after hatching. No jaws or beak develop. A few days after hatching a pair of tentacles develop on either side at the angles of the jaw; one of them may become bifurcated and each is provided with two channels through which the blood streams. External gills are not wholly wanting (as was formerly thought), for two days after hatching, when the operculum is commencing as a free fold, there are vascular lamellæ on the first three branchial arches. The gill-slits open later than the mouth; the hyoid cleft opens last by a tube into the first branchial cleft, a long way from its opening to the exterior. At the same time that the gill-slits open, a tufted filtering apparatus is developed from the branchial arches; this becomes vascular and must, it is thought, be respiratory, as no internal gills develop. The opercular folds do not cover the arms, but open by a slit on each side, anterior to the arm. The spiraculæ persist for a long time. The pronephros develops as in the ordinary frog. The vascular system develops as described by Maurer for *Rana esculenta*.

It is evident from the foregoing review that many points in the embryology of the Anura remain to be investigated; it is not, however, surprising that the work has been left undone, when we consider the geographical, the climatic, and other difficulties attendant upon making observations and collecting material.

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'87 SMITH, HERBERT H. On Oviposition and Nursing in the Batrachian Species Dendrobates. *Am. Nat.* Vol. xxi, p. 307.

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'77 SPENGEL. Die Fortpflanzung des Rhinoderma darwinii. *Zeitschr. wiss. Zool.* Bd. xxix, p. 495. (Translation of Espada, **'72**.)

'46* STEENSTRUP. Rapport sur l'histoire naturelle de quelques Amphibes de Danemark. Kiel. 1846.

'42 VOGT, CARL. Untersuchungen über die Entwickelungsgeschichte der Geburtshelferkröte, Alytes obstetricans. 1842, p. 87. Solothurn.

33 WAGLER. Descriptiones et Icones Amphib. Pt. 2, p. 11. 1833.

54a WEINLAND, F. D. Ueber den Beutelfrosch. *Arch. f. Anat. u. Phys.* Bd. xxi, p. 449.

'54b —— Lichtenstein's Remarks on Specimen of Notodelphys in Weinland's Collection. *Berliner Monatsber.* 1854, p. 372.

'98 WERNER, FRZ. Ueber Brutpflege bei Amphibien. *Verh. d. k. k. zool.-bot. Ges. Wien.* Bd. xlviii, Heft 1, p. 11.

'77 WILDER, B. G. Note on Embryo of Pipa americana. *Am. Nat.* Vol. xi, p. 491.

'90 WOLTERSTORFF, W. Vollständige Entwickelung eines Frosches (Hylodes?) im Ei. *Jahresber. u. Abh. Naturf. Ver. Magdeburg.* 1890, p. 317.

'54a —— WYMAN, JEFFRIES. On the Development of Pipa americana. *Proc. Boston Soc. Nat. Hist.* Vol. v, p. 13.

'54b —— Observations on the Surinam Toad, Pipa americana. *Amer. Journ. of Sci.* Vol. xvii, p. 369.

'57 —— On the Exhibit from Surinam, Hylodes lineatus. *Proc. Boston Soc. Nat. Hist.* Vol. vi, p. 269.

'59 —— On Some Unusual Modes of Gestation. *Amer. Journ. of Sci.* Vol. xxvii, p. 5.

'62 —— Dactylethra. *Proc. Boston Soc. Nat. Hist.* Vol. ix, p. 155.

APPENDIX I.

Since this paper went to press, there have appeared the following notes on Nototrema by Brandes, '99. "Larven zweier Nototrema-Arten. Demonstration." *Verhandlung der deutschen Zool. Ges., 9. Jahresvers.* Hamburg, p. 288.

The female of *Nototrema marsupiatum* carries a great number of small eggs (200). The full-grown larva has a rudder-tail with a well developed capillary network, hind legs in the condition of knobs, horny teeth, and an operculum with a lateral spiraculum. Through the spiraculum project two filaments, which can be traced to the first and second gill arches of the opposite side. The filaments are expanded at their ends into a delicate membrane, spread out under the egg-membrane. The gills are thus reduced to two, both arising from the same side. In the larva examined, the yolk had been entirely absorbed, so that life in the egg-membrane was no longer possible. A larva caught in water resembled a tadpole of the European Pelobates shortly before metamorphosis.

Nototrema oviferum carries only thirty large eggs. The tadpoles observed had well developed hind legs; the tails had entirely degenerated; there was no spiraculum, but through a broad slit in the operculum projected the two bell-gills described by Weinland ('54a). Four gill arches were present, and the gill filaments were attached to the first and second arches.

APPENDIX II.

Index to species, with a summary of references to each.

Alytes obstetricans. Europe.
Demours, 1741. Wagler, '33. Vogt, '42. Steenstrup, '46. Claus, '66. De l'Isle, '76. Leydig, '77. Gasser, '82. Hartmann, '96. Wyman, '59. Nature, '77. Bronn, '78. Knauer, '78. Cope, '85. Sarasin, '87. Boulenger, '86a. *Ib.*, '98. Werner, '98. Page **696**.

Amphignathodon guentheri. Tropical Andes.
Cope, '85. Page **700**.

Arthroleptis seychellensis. Seychelles.
Brauer, '98. Page **704**.

Chiromantis guiniensis (*C. refuscens*). West Africa.
Peters, '75. *Ib.*, '76 (plate). Ihering, '86. Nature, '77. Bronn, '78. Cope, '85. Sarasin, '87. Boulenger, '86a. Werner, '98. Page **689**.

Cystignathus gracilis (*Paludicola gracilis*). d'Orbigny, '47. Hensel, '67b. Sarasin, '87. Boulenger, '86a. Brazil, Argentine Republic. Page **691**.

Cystignathus mystaceus. Rio Grande do Sul.
Hensel, '67a. *Ib.*, '67b. Nature, '77. Knauer, '78. Bronn, '78. Sarasin, '87. Boulenger, '86a. Page **692**.

Cystignathus typhonius. Porto Rico.
Peters, '76. Page **692**.

Dactylethra capensis (*Xenopus lævis*). Southern Africa.
Wyman, '62. Gray, '64a. *Ib.*, '64b. Parker, '77. Boulenger, '81. Leslie, '90. Beddard, '94. Page **707**.

Dendrobates braccatus. West Brazil.
Smith, '87. Cope, '87. Boulenger, '88a. *Ib.*, '88c. *Ib.*, '95. (Boulenger refers indiscriminately to Kappler, '85, and to Smith, '87, and Cope, '87, although these authors have described two species, which Cope says differ in size.) Cope, '89. Werner, '98. Page **703**.

Dendrobates trivittatus. Dutch Guiana.
Wyman, '57. *Ib.*, '59 (under the name of *Hylodes lineatus*). Kappler, '85. Boulenger, '88a. *Ib.*, '88c. *Ib.*, '95. Smith, '87 (in which mention is made of a frog from the Santarem forest, which is perhaps *Dendrobates trivittatus*). Werner, '98. Page **703**.

Engystoma ovale. Paraguay.
Budgett, '99. Page **702**.

Hyla abbreviata (*Ololygon abbreviatus*). Rio Grande do Sul.
Hensel, '67a. Knauer, '78. Page **693**.

Hyla faber. Rio de Janeiro. (Early accounts under the name of *Cystignathus ocellatus.* Rio Grande do Sul.)
Hensel, '67a. *Ib.*, '67b. Goeldi, '95. Bronn, '78. Knauer, '78. Boulenger, '86a. Schönichen, '97. Page **692**.

Hyla goeldii. Rio de Janeiro.
Fritz Müller, '79. Goeldi, '95. Boulenger, '95. Werner, '98. Page **701**.

Hyla nebulosa. Rio de Janeiro.
Goeldi, '95. Page **691**.

Hylella platycephala. Southern Mexico.
Cope, '85. Page **706**.

Hylodes lineatus (?). Peru.
Wolterstorff, '90. Page **706**.

Hylodes martinicensis. West Indies.
Steenstrup, '46. Bello, '71. Bavay, '73a to e. Peters, '76. Selenka, '82. Andrews, '92. Nature, '77. Bronn, '78. Cope, '85. Sarasin, '87. Boulenger, '86a. Page **704**.

Lythodites latrans. Western Texas.
Cope, '85. Page **702**.

Nototrema fissipes. Brazil.
Boulenger, '88b. Page **699**.

Nototrema marsupiatum. Ecuador, Peru.
Boulenger, '80. Weinland, '54a. Nature, '77. Cope, '85. Sarasin, '87. Boulenger, '86a. Page **700**.

Nototrema oviferum (*Notodelphys oviferum*. *Opisthodelphys oviferum*). Venezuela.
Weinland, '54a. *Ib.*, '54b. Berthold, '56. Wyman, '57. *Ib.*, '59. Owen, '66. De l'Isle, '76. Nature, '77. Bronn, '78. Boulenger, '82. Cope, '85. Howes, '88. Sarasin, '87. Boulenger, '86a. Page **698**.

Nototrema plumbeum.
Werner, '98. Page **700**.

Nototrema pygmæum. Puerto Cabella, Venezuela.
Werner, '98. Page **700**.

Nototrema testitudineum. Ecuador. Peru.
Boulenger, '82. Cope, '85. Sarasin, '87. Boulenger, '86a. Page **700**.

Paludicola fuscomaculata. Paraguay.
Budgett, '99. Page **702**.

Phryniscus nigricans. Paraguay.
Budgett, '99. Page **702**.

Phyllobates trinitatus. Trinidad. Venezuela.
Boulenger, '95. Werner, '98. Page **703**.

Phyllomedusa hypochondrialis. Paraguay.
Budgett, '99. Page **690**.

Phyllomedusa iheringii. Rio Grande.
Ihering, '86. Knauer, '78. Howes, '88. Sarasin, '87. Goeldi, '95. Werner, '98. Page **690**.

Pipa americana. British and Dutch Guiana.
Fermin, 1765a. *Ib.*, 1765b. Laurenti, 1768. Göze, 1776. Spallanzani, 1785. Camper, 1788. Wyman, '54a. *Ib.*, '54b. Parker, '77. Wilder, '77. Sclater, '95. Leydig, '96. Bartlett, '96. Duméril et Bibron, '54. Wyman, '57. Peters, '76. Nature, '77. Knauer, '78. Bronn, '78. Boulenger, '86a. Schönichen, '97. Werner, '98. Page **693**.

Polypedates maculatus. Ceylon.
Gunther, '76. Ferguson, '76. Sarasin, '87. Page **693**.

Rana opisthodon. Solomon Islands.
Boulenger, '86b. Sarasin, '87. Boulenger, '86a. Page **706**.

Rana temporalis. Ceylon.
Sarasin, '87. Page **693**.

Rhacophorus eques. Ceylon.
Sarasin, '87. Page **691**.

Rhacophorus reticulatus (*Polypedates reticulatus*). Ceylon.
Günther, '76. Ferguson, '76. Bronn, '78. Boulenger, '86a. Page **701**.

Rhacophorus schlegelii. Japan.
Holland, '89. Ikeda, '97. Werner, '98. Page **689**.

Rhinoderma darwinii. Chile.
Gay, '36. Espada, '72. Spengel, '77. Howes, '88. Bronn, '78. Boulenger, '86a. Plate, '97. Werner, '98. Page **700**.

Spea bombifrons. Idaho.
Cope, '85. Page **702**.

Spea hammondii. Western North America.
Cope, '85. Page **701**.

EXPERIMENTS ON CUTTING OFF PARTS OF THE COTYLEDONS OF PEA AND NASTURTIUM SEEDS.

ABEGAIL C. DIMON.

The experiments to be described were undertaken as bearing upon the general problem of the relation of food supply to growth. They were carried on under the guidance of Professor T. H. Morgan, to whom I am much indebted for aid and suggestions. The variations in food supply were produced in the pea and nasturtium, both dicotyledonous plants, by cutting off part of the cotyledons, thereby reducing the amount of food stored up by the parent plant for the use of the seedling. The questions that arise relate to the effect upon the size of the seedling, upon the differentiation of its organs, and upon the number and size of the component cells, caused by thus reducing the food supply. These questions may be answered from the results of the experiments.

As already stated, the pea and the nasturtium were the plants selected. Before deciding on them, however, the morning-glory, sweet-pea, radish, common bean, buckwheat, mustard, cucumber, and pumpkin were tested as to their suitability, by planting specimens of each with portions of their cotyledons cut off. It was found that the peas and nasturtiums, possessing large cotyledons, were more easily manipulated, and that their seedlings were hardier than those of the other plants. Their seedlings, moreover, grew rapidly, so that differences in the relative size of the plants were early noticeable and were well marked. Under favorable conditions, however, good results might be obtained from some of the other species, and it would be interesting to see to what extent they corroborate those from the two plants here discussed.

The seeds tested for availability, and subsequently all the pea and nasturtium seeds, were treated as follows : After they

had been soaked in water for from twelve to twenty-four hours to soften them, their seed-coats were removed and, from some of the seeds, parts of the cotyledons were cut off with a sharp scalpel, while the others were left in their normal condition save for the removal of their seed-coats. The normal seeds underwent the soaking in water and removal of the seed-coats to make their condition like that of the others except in the one point of food supply. When thus prepared, all these seeds were planted on sawdust, which was kept wet during their growth. In the case of the pea and nasturtium, as soon as the plants from these seeds were well started a second lot of normal seeds, treated in the same way as the normal seeds described above, was planted. In a short time, usually about two weeks, the plants of the second lot were found to be about the same size as those of the first lot that had come from the reduced seeds. Sections of the stems were then cut freehand, and camera drawings made.

The amount of cotyledon cut off from the seeds varied very considerably, and was not in all cases quantitatively determined. The variation may be seen from Table I, where are

Table I.

Part by Weight left, expressed in Percentages.	No. of Peas.	No. of Nasturtiums.	Part by Weight left, expressed in Percentages.	No. of Peas.	No. of Nasturtiums.
10–15	—	2	31–35	14	5
16–20	10	9	36–40	5	1
21–25	8	9	41–45	3	1
26–30	16	8	46–50	1	—

represented the percentages by weight of the part left in the case of 57 peas and 35 nasturtiums. The seeds were weighed after the removal of the seed-coats and again after the removal of part of the cotyledons, and the percentages express the ratio between the two weights. The percentages in the case of the peas vary from 16 to 48, with more than half the individuals between 26% and 35% ; those in the case

of the nasturtiums vary from 10 to 41, with more than half the individuals between 16% and 25%, and nearly three-quarters between 16% and 30%. The chances, then, that from 65% to 74% of the bulk of any pea seed has been removed, are even ; and the chances are three to one that from 70% to 84% of any nasturtium seed has been removed.

The variation in the amount of cotyledon removed appeared to influence the rate of growth of the seedling, but the number of plants of which a quantitative record of the development was kept was too small to justify an attempt to lay down a rule concerning the extent of this influence. The seedlings will therefore be regarded as belonging to only two classes, the normal and the dwarf, the latter composed of plants growing from seeds that have been reduced by removing part of their cotyledons. Plants of the two classes sprouted at about the same time, and for a short time the differences between them were not striking. As soon, however, as leaves began to develop, the normal seedlings shot ahead, surpassing the dwarf seedlings not only in size, but also in the number and size of their leaves. A comparison of two groups of pea plants, as given in Table II, from readings taken at three different times, shows the relative rate of development. The figures represent the height of the plants in millimeters and their number of leaves, while at the foot of each column is placed the average of the readings in that column. The readings from the dwarf seeds are arranged in the order of the fraction of the seed that is used for planting, and the readings from the normal seeds, in the order of the weight of the seeds, the smallest fraction and the smallest weight being at the top of their respective columns. The first period, two weeks after the seeds were planted, corresponds to an early stage of development ; the second, five weeks old, is the stage just before the production of flowers by the normal plant ; and the third period, nine weeks old, is the period of maturity, when the plants are bearing flowers and seeds. The letters *fl.* and the word *pod* in the column marked "leaves," mean that the plant against which they are placed has a flower or a seed-pod.

TABLE II.

	TWO WEEKS.				FIVE WEEKS.				NINE WEEKS.			
	Dwarf.		*Normal.*		*Dwarf.*		*Normal.*		*Dwarf.*		*Normal.*	
	mm.	Leaves.	mm.	Leaves.	mm.	Leaves.	mm.	Leaves.	mm.		mm.	
a	17	5	35	11	22	9	77	23	35	—	95	fl.
b	10	—	29	11	29	9	70	19	47	—	89	—
c	16	5	32	13	35	15	86	27	44	—	108	pod.
d	22	7	32	11	26	9	77	27	35	—	102	fl.
e	26	11	32	11	54	20	89	27	70	fl.	117	pod.
f	26	9	38	13	47	17	77	27	63	fl.	95	—
g	29	9	41	13	60	21	92	28 fl.	77	pod.	108	pod.
Av. . . .	22	7	34	12	39	14	81	25	53		102	

From Table II we may collect several facts as to the relations in size and development between dwarf and normal plants. In the first place, the ratio of height of dwarf to height of normal, expressed in percentages, varies as follows: At two weeks, 65%; at five weeks, 36%; at nine weeks, 52%. The ratios between the number of leaves of the dwarf and of the normal are 58% and 56% for the two and five weeks old plants, respectively. No readings were made of the number of leaves at the third stage, because some of the plants had lost leaves, and the oldest leaves of all the plants were beginning to wither, so that the number had not as much significance as in the earlier stages. Similar measurements of height and number of leaves made on another lot of peas showed similar variations in ratios, so we may consider the readings here given as typical. The advantage in size, then, which the normal seedlings show in the first stage becomes more marked as the plants grow older, while the differentiation, so far as expressed by the number of leaves, though markedly greater in the normal than in the dwarf plant, does not increase much faster in proportion in the former than in the latter. The time of the first appearance of flowers is, however, more significant of the differentiation of the plant than is the number of leaves, for the latter may depend on the size of the plant, as well as on its degree of development. The time of flowering was unmistakably earlier in the normal plants, for the first flower appeared upon them at five weeks, whereas the first flowers appeared on the dwarf plants at eight weeks. At this time three dwarf plants blossomed, while during the same period five of the normal plants had borne flowers, of which three had developed into pods. These facts suggest the conclusion that the normal plants not only are larger than are the dwarf plants but develop more quickly than do the latter.

It remains, therefore, to be seen whether the foregoing conclusions to which the macroscopic examination has led are supported by the examinations of sections under the microscope. An examination of this sort may be looked to to answer the following four questions:

A. What is the relative size of the cross-section of a normal and of a dwarf plant?

B. What is the relation between the number of cells in a normal cross-section and a dwarf one?

C. What is the relation between a normal and a dwarf plant, as regards the size of the cells?

D. How does the degree of differentiation of the normal compare with that of the dwarf plant?

Attempts were made to compare sections of the dwarf seedling under the microscope with sections of a normal seedling of the same size, as a check, as well as with the normal seedling of the same age, but the only specimens of check seedling available for comparison were so much larger than the dwarf that allowance must in every case be made for a discrepancy. The cross-sections studied were all cut free-hand from a level less than an inch from the ground in the growing plant.

The first question in the preceding paragraph relates to the relative size of the cross-section of the stem of the normal and dwarf plants. In the cross-sections represented by the figures the diameters of the peas have the relative values of 36 and 53, or the diameter of the dwarf pea is .68 as great as the diameter of the normal; the diameters of the nasturtiums have the relative values of 30 and 38, or the diameter of the dwarf is .79 as great as that of the normal. Cross-sections of the stem of the check plants have the values 46 and 33 for the diameters of the pea and nasturtium, respectively. The dwarf and normal plants were five weeks old and the check plant two and one-half weeks old. The plants selected for examination were typical ones, and the fact that the ratio between the diameters does not correspond to the average ratios between the heights given in Table II may be explained in two ways:

1. The ratio between the heights, as was seen, decreases with the increasing development of the plants, and the degree of development of the plants from which the cross-sections were taken was probably not the same as that of the plants measured in Table II.

2. The stem of small plants is always thicker in proportion to the size of the plant than that of large plants, so less difference in cross-section than in height is to be expected. Interpret this discrepancy as we may, the fact remains that the stem of a normal plant has a greater diameter than the stem of a plant sprung from a seed part of whose cotyledons has been cut off.

The ratio between the number of cells in a normal cross-section and a dwarf cross-section can be determined by counting the number in a definite sector of each. The results of counting the number of cells in a sector of 30° are : in the normal pea (Fig. 1), 410 cells ; in the dwarf pea (Fig. 2), 311 cells ; in the normal nasturtium (Fig. 3), 223 cells ; in the dwarf nasturtium (Fig. 4), 208 cells ; in the check pea, 404 cells ; in the check nasturtium, 219 cells. The dwarf plant has, therefore, decidedly fewer cells than the normal ; in the case of the pea .76 as many, and in the case of the nasturtium .93 as many. If the ratio between the number of cells was the same as the ratio between the diameters of the cross-sections, it would mean that the cells must be of the same size ; since the former ratio is larger for both pea and nasturtium, it means that the cells of the normal are larger than those of the dwarf plant.

The conclusion as to the size of cells in dwarf and normal plants may be confirmed directly by counting the number of cells in a definite area. Proceeding to do this for Parenchyma cells, the following results were obtained: In the normal pea (Fig. 1), 22 cells ; in the dwarf pea (Fig. 2), 43 cells ; in the normal nasturtium (Fig. 3), 21 cells ; in the dwarf nasturtium (Fig. 4), 32 cells ; in the check pea, 50 cells ; in the check nasturtium, 29 cells. The regions counted were all in the same part of the stem, and other counts were made that corroborate the figures here given. These figures confirm the conclusion reached in the preceding paragraph as to the size of the cells in normal and dwarf plants ; but in the case of the check pea plant it is found that the cells are smaller than those of the dwarf plant.

Though the statistics from microscopic examination that

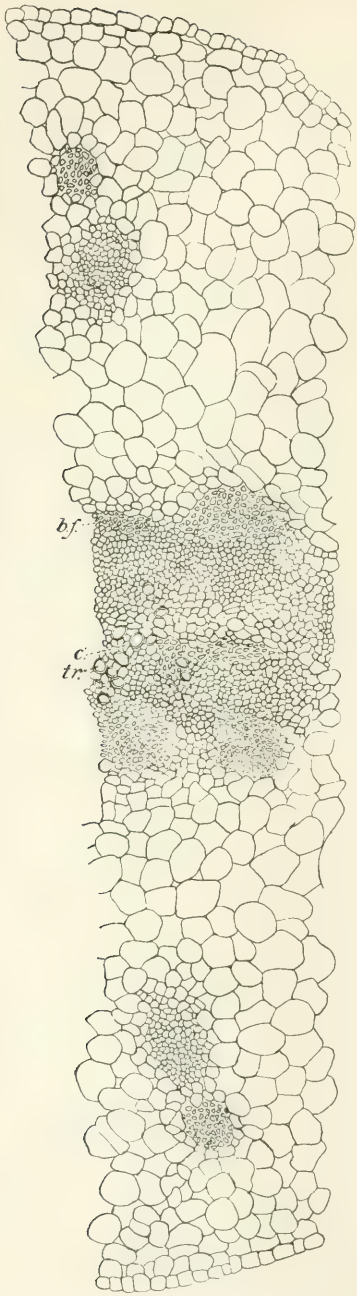

Fig. 1.

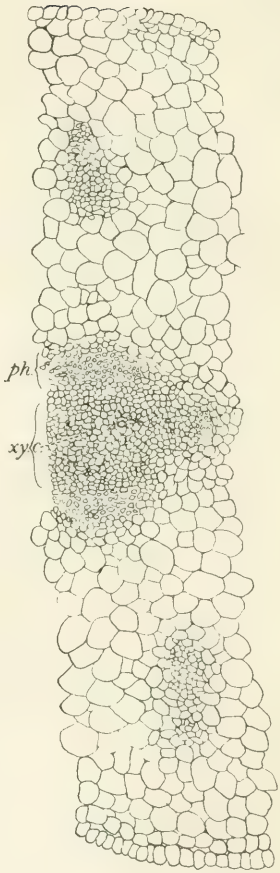

Fig. 2.

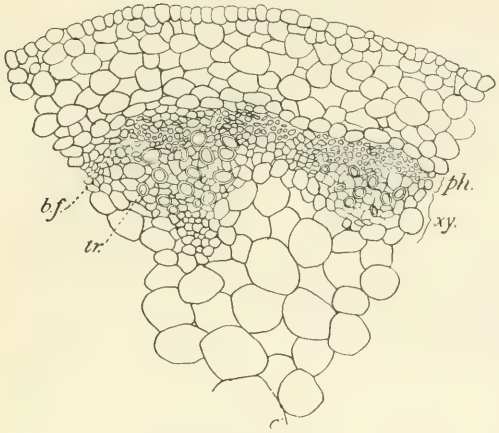

FIG. 3.

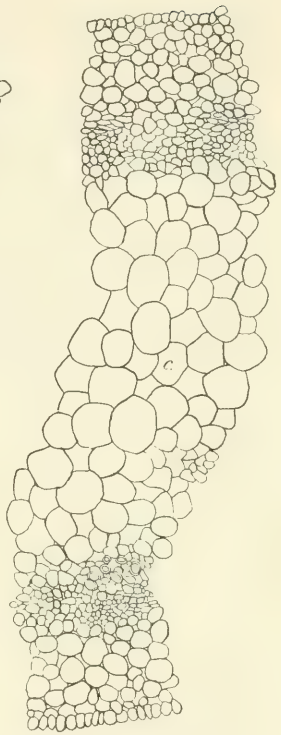

FIG. 4.

DESCRIPTION OF FIGURES.

b.f. = bast fibers. *tr.* = tracheid.
ph. = phloem. *xy.* = xylem.
c = center of stem.

FIG. 1. — Part of a cross-section of the stem of a normal pea seedling.

FIG. 2. — Part of a cross-section of the stem of a dwarf pea seedling.

FIG. 3. — Cross-section of a sector of the stem of a normal nasturtium seedling.

FIG. 4. — Part of a cross-section of the stem of a dwarf nasturtium seedling.

have been given and discussed were all drawn from three plants of each species, nevertheless that the general results are trustworthy is shown by another set of observations made on different pea plants. These gave the following results: Ratio of diameters of dwarf and normal plants, 111:62 (.56); cells in a given sector (7°), 165 in normal and 106 in dwarf (.64); cells in strips from center to circumference proportional in width to the size of the cross-section, 219 and 144 respectively (.66). The number of cells in a definite area was 29 in the normal and 44 in the dwarf. These measurements confirm those already discussed, for they show that the normal stem is larger in cross-section, is composed of a greater number of cells, and of larger cells than the dwarf.

The next question to be examined is the degree of differentiation of the various plants. This differentiation may be studied in the fibro-vascular bundles, where we may note the

appearance of the bundle as a whole, and the development of its elements. The appearance as a whole is indicated diagrammatically by the text-figures, which show especially marked differences in the case of the nasturtium. In the dwarf nasturtium the fibro-vascular elements are arranged in separate bundles around a central pith, while in the normal plant the phloem of the different bundles has run together, making a ring around the pith, the xylem being still discontinuous. Bast fibers seem highly developed with thick walls, and the tracheids are large, numerous, and clearly differentiated in the normal nasturtium stem; while in the dwarf stem the soft bast has just begun to show signs of thickening into fibers, and the tracheids are small, and comparatively few and poorly differentiated. In both specimens of pea the fibro-vascular

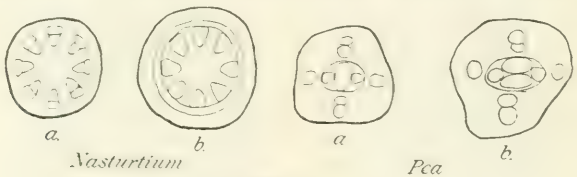

FIG. 5.

elements are arranged in an aggregate in the center of the stem and in four small groups peripheral to the large group. These bundles are more distinct and woody-looking to the naked eye in the normal than in the dwarf plants, and both bast fibers and tracheids are more numerous and highly differentiated. The normal plant, therefore, is more highly differentiated than the dwarf, as well as larger.

The conclusions reached from macroscopic and from microscopic examinations are then in accord with one another, and may be summarized by the statement that the removing of part of the cotyledons of a seed retards not merely the growth in size of the plant produced from that seed, but also its development. The plant, however, is not the counterpart of a younger normal plant, for it was found from comparing dwarf plants with check plants that the dwarf plant of a certain height was further developed than the check of the same height. The same point is illustrated by the fact that

a full-grown dwarf plant is smaller than a full-grown normal plant, as is shown by the nine-weeks stage of Table II.

The effect, it would seem, of removing a part of its food supply from the seed is not merely a transient one, but is one that can be traced through the whole life of the plant, and even increases as the plant grows older. The amount of food supply in the cotyledons influences, perhaps, the early stages of growth, while as the plant increases in size it becomes more and more vigorous and tends to grow more and more rapidly; so that a plant that is given an advantage over its fellow at the start will increase this advantage during subsequent development.

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THE PROBLEM OF DEVELOPMENT

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NO single problem in biology excites so wide an interest at the present time as that of organic development. During the greater part of the present century naturalists have been especially concerned with the historical or phylogenetic development, or evolution of the animals and plants found living at present on the earth's surface, but at the same time the development of the individual from the egg, or the ontogenetic development has attracted an ever increasing number of investigators. The discovery of a supposed parallel between the development of the individual from the egg, or the ontogeny, and the historical development, or phylogeny, of the species, has greatly excited the interest and the activity of students of embryology, and has withdrawn attention from other and, as I believe, more important problems of development.

The main object of the present essay is to contrast the results of the study of embryology as an historical problem, with the results of the study of embryology as a series of problems directly connected with the phenomena of development itself. The one I shall speak of as the historical problem, the other as the scien-

tific, although this distinction will not be generally admitted by embryologists, since it might seem to imply that the one method of study has greater value than the other.

PART I.

The early embryologists studied only the development of the chick, which has one of the most complicated forms of development that we know. It was from a study of the development of the chick, by such men as Harvey, Wolff, Pander, and Von Baer, that many of the problems of development were suggested. It has been, however, only during the latter part of the present century that a systematic study has been carried out in all the different groups of the animal and plant kingdoms. An enormous accumulation of information has been the result. From time to time attempts have been made to classify these observations in such a way that so-called principles or laws of development might be formulated.

Let us, in the first place, examine some of these discoveries and see how students of embryology have attempted to interpret their results.

One of the first steps in the development of all eggs is connected with peculiar changes that take place in the nucleus of the egg. The nucleus is a spherical body lying in most cases at, or near, the centre of the egg. It has a definite wall, and contains in its interior a fluid and a more solid meshwork of a substance called chromatin. For reasons that will appear later the chromatin is looked upon as the most important element in the nucleus, and even, according to many investigators, in the egg itself. The changes that take place at the beginning of development are concerned especially with the chromatin of the nucleus.

It has been found, for instance, that the first noticeable change in the egg is the partial dissolution of the nucleus; all that

remains of the nucleus are its threads of chromatin, and these separate into a number of pieces, or rods, called the chromosomes. It has been discovered that for each egg there is a fixed number of chromosomes, and, moreover, that this number is always exactly half that found in the cells of the body of the animal; or expressed more concisely, the number of chromosomes in the egg is half that characteristic of the somatic (body) cells.

Around the chromosomes set free in the egg there is formed from the protoplasm of the egg a spindle-shaped structure composed of threads, or fibres. These fibres become attached to the chromosomes, and the latter come to lie in the equator of the spindle. At each pole of the spindle there is generally found a small body, that can be deeply stained. It is called the centrosome. Around the centrosome other fibres or rays extend out into the egg, forming, as it were, a halo around each pole of the spindle. The spindle moves to the surface of the egg, and turns so that one pole lies at the surface. At this time each of the chromosomes, that still lie at the equator of the spindle, divides into two equal parts, and the parts begin to move away from each other towards their respective poles of the spindle. The outer end of the spindle now extends into a small protrusion of the egg, and this protrusion pinches off and carries with it the contained chromosomes. Thus half of the chromosomes, formed by division of those that first appear in the egg, are extruded from the egg in the first polar body, and the egg also loses the outer half of the first spindle, as well as a certain, small amount of its protoplasmic substance.

The next changes lead to the formation of the second polar body. Around the chromosomes that remain in the egg a new spindle, very similar to the first one, is formed. The chromosomes, that were left in the egg, come to lie in the equator of the new spindle, and there they divide again into equal parts, and

the halves migrate towards their respective poles. The spindle turns so that it lies with one pole at the surface, and at this point the protoplasm of the egg forms again a protrusion into which the outer part of the spindle is carried. The chromosomes migrate towards the poles of the spindle, and the protrusion pinches off to form the second polar body. The second polar body contains half of the amount of chromatin left in the egg after the extrusion of the first polar body.

It will be seen that the egg has lost three fourths of the volume of its chromatin but, since in each spindle the chromosomes divided into halves, the same *number* of chromosomes remain in the egg that were present at the beginning.

At about the time of the extrusion of the polar bodies the spermatozoön of the male enters the egg. It consists of a dense mass of chromatin forming the head, and a long, vibratile, protoplasmic tail. The tail is left at the surface of the egg, or is absorbed by the egg and disappears. The head swells up, and forms a spherical nucleus, the male nucleus. It is known that this male nucleus contains the same number of chromosomes characteristic of the egg, and hence also half the number of chromosomes found in the body-cells of the male.

The chromatin left in the egg after the extrusion of the second polar body also forms a nucleus, the female nucleus, and the male and female nuclei now sink deeper in the egg, and also move towards each other, and finally fuse into a single nucleus, the so-called segmentation nucleus.

These changes that have taken place in the egg are known as the maturation and fertilization of the egg; they have been fully made out only during the last fifteen years; although the fertilization of the egg by the spermatozoön has been known since 1786, and the extrusion of the polar bodies was first observed in 1848. For some time it was supposed that the entire nucleus of the egg was

thrown out into the polar bodies, and a new nucleus brought in by the spermatozoön; but we now know that the egg and the spermatozoön contribute an equal number of chromosomes to the segmentation nucleus.

Very great interest has been aroused in these changes that lead to the extrusion from the egg of three fourths of its amount of chromatin; and much speculation has been indulged in with regard to the meaning of the phenomenon. Some embryologists have attempted to give a physiological explanation to the process, while others have given an historical interpretation. Minot, Van Beneden, and Balfour have supposed that the egg contains at first the properties of both male and female elements, and that by the extrusion of the male element, in the two polar bodies, the egg is prepared to receive the spermatozoön. But aside from the fact that we should suppose one polar body would be sufficient to set free the male element, and that we know of no similar process by means of which the spermatozoön frees itself of its female element, there are many more recent observations and experiments that put the matter in an entirely different light.

Strasburger's idea that the essential element of the nucleus, the idioplasm, is reduced to one half, and must be renewed by the material received from the spermatozoön, is also undoubtedly a wrong interpretation, as subsequent work has shown; for we now know that the female and the male nucleus if supplied with protoplasm are each capable of forming a complete embryo.

Weismann has also attempted to give a physiological interpretation of the process of extrusion of the polar bodies. He supposed, at first, that the egg freed itself by means of the two polar bodies of that part of the chromatin-substance that had been concerned with the building up of the egg itself while it was still a part of the parent organism. Later, Weismann supposed that while in one of the polar bodies the ovogenetic substance was set

free, in the formation of the other a qualitative division of a different sort takes place in the chromatin. In order to understand fully Weismann's idea, it is necessary to know something of the way in which he supposes the qualities of the adult are contained in the chromatin of the egg. His view may be briefly stated as follows: every part of the embryo and of the adult is represented by certain elements,—the determinants in the chromatin of the egg. These, he supposes, have the property of dividing, and as a result of increasing in number. One of the divisions of the chromatin of the egg that leads to the formation of one of the polar bodies is a qualitative division in the sense that the egg loses half of its determinants, which are replaced *in kind* by those received from the male. The other division of the egg-nucleus to form the first polar body, Weismann believes, as has been stated above, to be a division by means of which the egg loses that part of its chromatic material that was needed to form the egg itself,—the ovogenetic chromatin.

Since the egg-nucleus after the extrusion of the polar bodies is equivalent to the sperm-nucleus, we should expect to find some process in the formation of the spermatozoön equivalent physiologically to that of the maturation of the egg, but no such process is known; and Weismann found himself unable to give a satisfactory account of its absence. In fact, we can now state definitely that in the development of the spermatozoön there is no process that can be interpreted as Weismann has interpreted the first polar body; and there are certain facts in the development of both the spermatozoön and the egg that show Weismann's views to be incorrect.

In regard to the interpretation of the second polar body, it is more difficult to give a demonstration of the incorrectness of Weismann's explanation; but there are, at least, certain facts that make the view improbable in its physiological sense; for even if

it should prove true that half of the chromosomes are set free at the second (or even at the first) division, it by no means follows that the egg has lost half of its inherited qualities.

Weismann's entire conception of the origin of variation, and hence of the possibility of natural selection, is based on his idea of the qualitative division of the chromatin of the egg and of the spermatozoön at one stage in their development. He supposes new variations to arise by means of the different combinations of male and female chromosomes at the time of fusion of the male and female nuclei. Weismann even goes further, and maintains that *the purpose* of the supposed qualitative division is to produce new variations. He thus introduces a teleological element into the problem, under the disguise of its being the outcome of natural selection. In other words, natural selection has fixed and made constant a change that has for *its purpose* the bringing about of new variations for the further action of natural selection !

Aside from the uncertainty of the reasoning there are facts, that make, at least, improbable Weismann's teleo-physiological explanation ; and these will be apparent as soon as some further observations and experiments have been described.

Several naturalists have maintained that each polar body is equivalent to the egg itself, and that the process of division is a quantitative process except in so far as the amount of protoplasm is concerned. A careful comparison of the maturation of the egg and of the spermatozoön has shown that the egg and its polar bodies (after the division of the first polar body that generally takes place) are equivalent to the four spermatozoa that develop from a single mother-cell. Every spermatozoön is capable of fertilizing an egg, i. e., they are all equivalent. If, then, the process by which they form is the same as that by means of which the

polar bodies develop, we should expect to find each polar body also equivalent to the egg or to a spermatozoön.

The most obvious difference between the egg and the polar bodies is in the relative amount of material contained in them; and the question arises as to whether the polar bodies might not develop into embryos if they contained more protoplasm. The observations of Francotti, that have been recently published, show, that in one animal at least,—a planarian,—the first polar body may, indeed, form an embryo.

Furthermore, an experiment, first carried out by Boveri, has shown that each spermatozoön, if supplied with enough of the egg-protoplasm, may produce an embryo, even in the total absence of the egg-nucleus. By shaking violently in a small tube the unfertilized eggs of the sea-urchin they may be broken up into pieces of all sizes, some of which will contain the original egg-nucleus, and some of which will not contain any nucleus. If a single spermatozoön enters the larger of these pieces without a nucleus, the piece begins to develop, and ultimately produces a perfect embryo. We thus see that it is not even necessary for the male to fuse with the female nucleus in order to form an embryo, but the spermatozoön can, if supplied with protoplasm, produce the complete form. That the egg also may develop without fertilization after the extrusion of the polar bodies, is shown by certain eggs that develop by parthenogenesis, and by the recent experiment of Loeb in which the eggs of the sea-urchin that have extruded both polar bodies develop when put into certain salt-solutions.

These recent observations and experiments show that Weismann was wrong in his interpretation of the first polar body; and, while it must still be admitted that possibly at one of the divisions half of the chromosomes may be lost, yet there are no facts known to support the idea that in this way half of the hereditary

qualities are set free, or separated; and there is some evidence to show that one chromosome is like another, and it would make no difference so far as the quality of the division is concerned whether half of each chromosome is lost at each division, or whether half of the chromosomes (assuming them to be double) are set free. It seems to me that Weismann has been most unfortunate in his attempt to find a physiological explanation of the maturation of the egg.

There is another point of view, the historical, from which we may consider the process of maturation. The two maturation divisions seem to be equivalent, as we have seen, to the last two divisions by means of which four spermatozoa are formed. A few embryologists interpret the division of the egg to form the polar bodies as the repetition of a process by means of which four eggs were once produced; each equivalent to one of the four spermatozoa, and each capable, after fertilization, of development. In the course of time one of the three eggs has come to retain all the yolk, and most of the protoplasm, and the others have correspondingly lost their material, and fail, in consequence, to develop. Or expressed in a different way, the process has no physiological significance at all except so far as the egg simply repeats an historical process. Should this interpretation turn out, in the end, to be the most probable one, then we have a good example of the uselessness of trying to interpret as useful all the facts of embryology.

At present it is the habit of embryologists to attempt to show that each change in development is useful, and hence exists. They attempt to give *a reason* showing that each historical change has been useful to the egg, but I do not know of any canon of science that requires us to put this interpretation on all natural phenomena.

This interpretation of the formation of the polar bodies as the

repetition of a past event by means of which four eggs were formed, vindicates the historical method, in the sense that we get a certain insight into the *meaning* of the process, that we would not otherwise have had; but we should, at the same time, not forget that we have not a causal explanation of the formation of the polar bodies by showing that, in the past, each polar body was an egg. All we can infer from this interpretation is that the egg has at present nearly the same structure, so far as concerns the first changes that take place, that it had in the past, when four eggs were produced; but its structure has also changed in so far that at each of the divisions, to form the polar bodies, one of the parts in each case now remains extremely small, but, except so far as their volume is concerned, the polar bodies retain all the physiological properties of eggs.

We may now pass to an examination of the stages of development that follow the fertilization of the egg. Around the nucleus, formed by the fusion of the male and female nuclei, a new spindle develops. The origin of this spindle is, at present, under discussion; and it is best not to speak dogmatically, but it seems very well established in some cases, that its origin is connected with the introduction of a body, or of a substance, into the egg with the spermatozoön. Not that the spindle itself is introduced, but an exciting substance that causes the egg to produce a spindle. On the other hand, it is equally certain that in many cases the egg itself without being fertilized can also produce a spindle as in parthenogenetic development. The nuclear wall now dissolves, and the chromosomes of the nucleus appear. We find as many chromosomes as were in the female nucleus plus those that were in the male nucleus. The number is now the same as that characteristic of the body-cells, and we have some evidence to suppose that the same chromosomes have reappeared that were present in the egg- and sperm-nucleus. Each divides into equal parts,

and the parts separate and move towards the poles; then the whole egg divides, generally also into equal parts.

To attempt to discuss the many problems connected with the division of the egg, would carry us into two special fields of study; but I may point out that we meet here, on the one side, the historical interpretation of the meaning of the cleavage, and, on the other side, a study of the phenomena of the mechanism by means of which the division is brought about.

The first observations on the division of the egg were made in the early part of the present century; but the process was not clearly understood until some time later. With the formulation of the cell-theory, in 1839, the question of the origin of the cells of the body arose; and it was soon suspected, that the division of the egg is a process that leads to the production of the cells of the adult; and the further conclusion was not long in being reached, that the egg itself is a cell, the mother-cell of the host of cells that make up the body of each individual.

The first division of the egg is succeeded by a second in each part, or cell; and then each of the four cells again divides; the process continuing until a large number of cells have been formed. These cells are usually regarded as the building blocks, out of which the embryo is formed; but the comparison is imperfect in one essential respect, inasmuch as the building blocks are not passively moved about by some external power, but they contain within themselves the power of movement and of producing in this way the embryo.

It is not easy to give in a few words a general idea of how the cells make the embryo, for there are many different ways in which this is brought about. In some cases plates or sheets are formed by the partial fusion of the cells; and these plates by bending, or folding, produce the different organs. In other cases solid growths are formed, and these mould themselves into the

organs; and there are still other ways in which the organs may develop. We can not, however, pass over this period of the development without saying a few words about the germ-layer hypothesis, and the gastræa theory.

Casper Friedrich Wolff first discovered, in 1759, that the embryo of the chick was formed out of two layers or sheets; and later embryologists discovered another layer, itself composed of two layers, lying between the outer and the inner layers of Wolff. Huxley, in 1849, drew attention to the similarity between the structure of some of the lower animals—the hydroids and jellyfish (whose body is composed of an outer and an inner layer of cells) and certain stages passed through by many of the higher forms, in which an outer layer and an inner layer are present. This idea of a similarity between the two series was later elaborated into an hypothesis that affirmed an historical connection between the two. It was stated that in the development of the higher forms a two-layered stage is passed through, which is an historical repetition of the adult hydroid, from which the higher forms are supposed to have been derived. The outer layer or ectoderm of the hydroid is said, therefore, to be *homologous* with the outer layer or ectoderm of the embryo of the higher forms, and the inner layer of the hydroid that lines the digestive tract, is said to be homologous with the inner layer of the embryo that forms later the wall of the digestive tract of the adult. As a corollary to these statements it is added that the ectoderm of one embryo is homologous with that of any other; and the same holds true for the endoderm, and even for the middle layer or mesoderm. If it could be positively established that there really exists such an historical connection between the different embryos and the older forms, the results of this comparison might have a real meaning, even if they did not have any further value; but it soon became evident that the comparison

was perilous in the extreme, and wide differences of opinion arose in regard to the interpretation of the same facts; and in several cases it was discovered that what becomes the outer layer in one form becomes the inner layer in a related form. Furthermore, on closer analysis, it will be noticed that the idea of an homology, based on an historical connection, is not so simple as appears at first sight, for the connection is broken in each generation. The individual begins as a single cell, an egg. This, after fusion with another cell derived from another individual, the spermatozoön passes through a series of stages to reach the adult form. Admitting that there has been an organic continuity between the ancestral adult form and the egg of the last generation, and even admitting a similarity between the two, it is still by no means clear, from a causal point of view, that the former could have had any influence on the latter. Put in this way,—the usual formula,—the statement is, I think, misleading; but, as I shall attempt to show later, the similarity of the embryo to its ancestor may be accounted for in such a way that at least nothing mysterious, or metaphysical, is included in the statement.

The crowning point of the germ-layer hypothesis is to be found in Haeckel's gastræa theory. The theory states that all the higher animals have descended from an ancestral form, whose body was made up of two layers, an outer and an inner. At one end of the body is the mouth opening, and at this point the two layers are continuous with each other. This ancestral form is repeated in the development of all the higher forms. Haeckel does not hesitate to affirm that there exists a causal connection between the ancestor and the embryo, as the following quotation shows :—

"I have represented the paleontological history of the species, the phylo-

geny, or the genealogy, as the real cause, on whose action the entire developmental history of the individual, the ontogeny, or embryology, rests. Without the former the latter could not exist. The cardinal point of this relation is that the connection between the two is a mechanical-causal one. The ontogeny is a short repetition of the phylogeny brought about mechanically through the function of inheritance and adaptation."

We find in this series of affirmations a confusion of ideas that will appall any one who will try to think out how the phylogeny can be the mechanical cause of the ontogeny. It is one thing to state that this is the case, and quite a different matter to show, from a physical standpoint, not only that such a thing is possible but that it is also true. While we may admire the daring flight of imagination that conceived such an idea from the meagre facts, yet we can not forget that science is neither eloquence nor fiction.

In the last eight years a more elaborate development of the idea of homology has taken place. A detailed examination of the stages preceding the formation of the germ-layer periods has shown that, in a number of forms, there is a remarkable agreement in the method of division of the egg of many animals in the same group, and even in different groups. The resemblance is so close, and is carried out through such a number of complicated divisions, that it seems scarcely possible to maintain that the similarity is a mere coincidence. The embryologists who have, with great patience and skill, worked out the method of division of these eggs have been unanimous in their belief that there is a direct connection of some sort that must account for the resemblance. In at least four large groups of the animal kingdom, the Annelida, Mollusca, Turbellaria and Nemertea the method of division of the egg in many forms of each group has been found to be the same or nearly the same. Not one of the embryologists who have studied these eggs, has gone so far as to imagine that the results are to be interpreted as the repetition of

ancestral animals, that are represented at each stage in the division of the egg by the group of cells that we find in embryos at the present time. If we choose to indulge in such a fancy, there is nothing to prevent our doing so, in case we are willing to substitute imagination for evidence.

How, then, can we interpret the facts, if we reject the idea that the resemblances are not due merely to chance, and that they are not the repetition of an ancestral adult form? There is another alternative, the one commonly accepted; namely,—that the four groups in question have descended from a common ancestor in which the cleavage of the egg took place as we find it taking place at present in many of the living descendants. Let us examine more carefully this hypothesis, and see in what sense it offers us an explanation of the phenomena. The hypothesis includes, in the first place, the implication that the eggs of all these animals are parts of the same original protoplasm that has been handed down from generation to generation. The explanation rests on the assumption that the protoplasm has retained in many of the descendants the same structure, so far as the cleavage factors are concerned. It is this historical sequence, or connection, that is more or less satisfying to our minds, and if we like, we may call it an explanation, since it removes the element of chance and of mysticism from the phenomenon. On the other hand it will be noted that this view gives us no insight into the causes that produce the kind of cleavage in each egg, and it is in the study of these causes that science is chiefly interested. Further, it will be admitted that because there has been an organic continuity between the past forms and the present ones it does not follow that the division of the egg must necessarily be the same in two forms descended from a common form, even when the two are closely similar in adult structure; for we find in each of the four groups named above, that

some eggs divide entirely differently from the type-form, and, moreover, the form of the cleavage can be artificially changed by altering the conditions of those eggs that follow the typical method if left to themselves. Finally, it must be granted that the same type of cleavage might appear in a group that had no immediate historical connection with the four groups in question, as a result of the same factors or causes being present. Indeed it is by no means entirely settled that this may not be the case in some one or more of the four groups in question.

The same reasoning that has been followed here in the case of the dividing egg applies with equal force to the germ-layer hypothesis, and need not be repeated. To prevent misunderstanding, I must add, that the argument in favor of the two-layered condition of the embryo in all the higher groups being connected by an unbroken series of stages with the embryo of the same common ancestor, is *by no means so probable* as in the case of the dividing eggs, that we have just examined; for, since the condition itself is so simple, and develops in such a variety of ways, we can scarcely ascribe to this view any greater value than that of a pleasing invention that may have in some cases an historical foundation in the sense defined above.

We may now pass to an examination of the later stages of development. It will be well to confine our examination to a single group, the vertebrates, in which we can study to much greater advantage the historical problem of development.

The order in which the different groups of vertebrates appeared historically on the earth's surface is known, at least in its broadest aspects, and the evolution of the higher groups from forms like those in the lower ones is a matter of the greatest probability. When we find, therefore, in the embryos of the higher animals structures that are peculiar to the lower adult forms we have sufficient evidence to warrant a careful consideration of the facts. A few examples will suffice to illustrate my meaning.

In the development of the chick we find a solid cord of cells, the notochord, extending along the entire length of the embryo on the dorsal side beneath the nerve-cord. The simplest form of living vertebrate, amphioxus, has a similar notochord in the same position; and we find in all the forms standing between the chick and amphioxus the same notochord appearing in the embryo. The similarity of the cord in the different forms, and its presence in intermediate forms makes it highly probable that there is an historical connection between the series. Whether the notochord in the embryo of the chick is a repetition of the notochord of an adult form like amphioxus, or whether the notochord of the chick is to be compared only with the notochord in the embryo of amphioxus are questions that will be considered a little later. It may be added here, that the notochord appears as early in the embryo of amphioxus as in the chick, and, if comparisons are to be made, it may be well to keep this fact in view.

We find in later stages of development of the chick that an investment of middle-layer cells is formed around the notochord and nerve-cord, inclosing them in a continuous mantle; and somewhat later the notochord begins to disappear at intervals where it is encroached upon by the investing sheath, and the sheath develops into a cartilaginous structure that breaks up into a series of blocks or vertebræ. This is also the condition of the backbone of the cartilaginous fishes. In later stages bony tissue forms in the cartilage, and we have a condition like that in the amphibia which leads to that of the adult bird.

We find similar relations in other parts of the body of the embryo chick. The heart begins as a simple, straight tube beneath the pharynx, it then bends on itself, so that the posterior end is carried forward, and we see a stage not very different from the heart of the shark. Later, partitions appear in the tube, that divide it into right and left sides, but the partitions are, for some

time, incomplete, so that a double circulation does not exist, and this condition we find in some of the groups below the birds.

The most striking embryonic organs that bear a resemblance to the lower forms are the gill-slits and gill-arches with their contained aortic arches. There appears in the chick embryo during the second and third days of incubation a series of vertical slits on each side of the pharynx, and some of these open so that the cavity of the pharynx communicates, on each side of the neck with the exterior by means of the series of slits, or gill-clefts. In the arches between the slits we find blood-vessels, that lead from the anterior end of the heart, through the arches, to the dorsal side of the pharynx, where they empty into the dorsal blood-vessel. In later stages the gill-clefts disappear and most of the aortic arches. It is obvious that we have here appearing in the embryo of the bird, a system of organs exactly like that found in fishes and amphibians by means of which the blood in the gills is aërated by water taken in by the mouth and passed outwards through the gill-slits. The chick enclosed in its egg-shell, obviously can not make use of its gill-system, and we find in later stages that the gill-slits disappear. Almost all embryologists have compared this system in the chick with that of the fish and amphibian adult stages, and speak of the repetition in the chick of an ancestral stage through which the birds have passed. I wish, however, to draw attention to the fact, that in the embryos of both fish and amphibia we have gill-slits appearing at a very early stage, as early, in fact, as in the chick; and the comparison between these early stages in both series is just as obvious as between the stages in the embryo bird and the adult fish and amphibian.

One further illustration may be given. It is said that at one stage in the development of the chick the rudiments of teeth appear in the beak, but subsequently disappear. It is known from

fossil remains that at a period before the modern birds appeared there existed birds having reptilian teeth; and we also know that both fossil and living amphibia and fish have teeth. The appearance of teeth in the embryo-bird is generally interpreted as a repetition of the condition of the ancestral birds, but it will be obvious, that since only the beginning of teeth appear in the chick, we must compare them not with the teeth of the adult ancestor, but with the embryo of that form.

These illustrations will suffice to give an idea of the historical problem that we meet with in the development of the higher vertebrates. The more naïve interpretation of these facts is, as we have seen, that during development the embryo passes through a series of stages that are like the ancestors of the birds. Haeckel is the greatest living exponent of this extreme form of statement. Other embryologists are contented to state that in the development of the bird we find many structures like those of the ancestral forms from which the birds have descended; and nearly all embryologists add, as a corollary, that there are also present in the embryo-bird other structures that were not present in the ancestors, but are peculiar to the embryo; and even that the ancestral features may be entirely lost under certain conditions. Most of the embryological work of the last twenty-five years has been inspired with the idea of determining what characters of the embryos of each group are ancestral, and what are embryonic only. If any criterion could be found by means of which the two kinds of structures could be separated, then embryologists hoped to reconstruct the past from the embryos of living forms. It must be confessed that up to the present moment the results have been discouraging, as no certain criterion has been found. Leaving, however, on one side this possible deduction from the facts of embryology, let us address ourselves to a further examination of the relation between the embryo and the ancestral series of adult

forms. Can we not apply here also the interpretation that I have discussed in connection with the historical problems in the division of the egg? Can we infer from the facts of observation anything more than that since embryos of all the vertebrates have many similar structures they repeat a common embryonic, ancestral form? They are alike, on this hypothesis, because in the eggs the same factors are at work, and produce the same structures; and they will be different just in so far as other factors come in to change the action of those present at first. These changes may, moreover, come in the embryos of the lower forms as well as in those of the higher forms. From this point of view we need not discuss whether the gill-slits in the chick are useful or the reverse, since with them the chick is still able to produce a bird without gill-slits, and we can readily imagine how the same goal might be reached without the gill-slits appearing at all. The presence of the gill-slits in the embryo is accounted for on our hypothesis by the presence of similar factors in the egg of the bird and of the fish; and the historical side of the problem leads us to conclude that the presence of these same factors is connected with the common origin of the fish and the bird, or in other words that they are both parts of the same material that has been handed down from the past.

It is unquestionably true that most modern embryologists have not been content to limit their conclusions to these statements, but have preferred to draw the more striking inference; namely, that ancestral adult structures themselves are repeated. I have no desire to avoid this issue, but I think the facts do not warrant our drawing such a conclusion, and I cannot protest too strongly against the Haeckelian method of stating that the phylogenetic series gives us a "mechanical-causal" explanation of the embryology.

There is one further point that I have not touched upon. If

the similarity between the embryo of a higher form and of a lower form is carried out to a late stage in the development, then it may appear that the embryo of the higher form passes through the adult stage of the lower in so far as the late stage of the lower form is like the adult of that species. Hence, I think, may have arisen the usual statement, with its vague implications, that the individual development repeats the adult ancestral stages. If, however, we put the results in the form of statement that I have given above we remove, it seems to me, much of the mystical element of the so-called "biogenetic law," and have a legitimate interpretation of the similarity between the embryo of the higher form and the adult of a lower group, through the embryo of the latter. Von Baer, in 1828, drew the conclusion from his study of the development of the chick that the embryos of animals belonging to the same group are like each other and follow the same method of development. The more nearly two forms are related, so much later in the development will the embryos of these forms be alike. This statement will not, at the present time, cover the facts that have been discovered. In the first place we find that the same method of development, especially of the early stages, may occur in widely different groups as in the annelids, molluscs, and turbellarians. In the second place we have evidence showing that forms within the same group, and supposed to be more nearly related to other forms in the same group than to any other group, may differ much more in their mode of development than two forms from different groups. For example, the squid follows a method of development totally different from that of the fresh water mussel, while the latter in its development has a large number of points of resemblance to the embryo of a marine annelid.

But the most profound difference between Von Baer's hypothesis and the more modern view is in the recognition by the

latter of the similarities of the embryos being the outcome of a common descent. Moreover, the special deductions that I have drawn from the observations, that the resemblance is due to the same factors being present in both eggs, and the presence of these factors is connected with, but not necessitated by the historical continuity of the substance of the egg, carry us much further than does Von Baer's statement.

Another problem that is sometimes discussed is whether the eggs of two animals are at the beginning of development more like each other than are the adults, or whether they differ as much from each other as do the adults. A discussion of these two possibilities will not, I think, help the advance of embryology, but, on the contrary, will draw attention away from the real problems that need to be worked out at present; for, until we can find out how the structures of the adult arise from the egg, it is a waste of time to weigh the relative advantages of the two conceptions. We can relegate the problem to the limbo of metaphysics and leave its discussion to those who delight in making subtile distinctions.

PART II.

In the preceding pages the problem of development has been examined from its historical standpoint. Around the conclusions hang all the doubts of the historical method. Granting that present forms have come from others living in the past, and that the organic chain has never been broken, still the historical method can at best only decide as to what is in a given case the more probable view; and since it is beyond our power, either to reverse the process of evolution or to repeat it, there is no criterion by means of which we can decide with certainty in regard to any conclusion, however probable it may be. The need of a different method has made itself felt more and more, as embryologists

have become convinced of the incompleteness and uncertainty of the historical side of the problem.

Observation of the series of changes through which the egg passes to become the embryo is necessarily the first step, whatever method may be subsequently followed. As a result of direct observation we might infer something of the mechanism by means of which certain changes take place. For instance, the fibres of which the spindle of the egg is composed have been observed to shorten as the chromosomes separate; and it has been inferred that they are contractile and pull apart the chromosomes. Likewise the fibres that radiate from the poles of the spindle have been supposed to bring about the division of the egg. The folding of certain parts of the embryo after the germ-layers have formed has been ascribed to differences in the amount of water, or other fluids absorbed by certain parts, causing the layers to bend in or out according to the local conditions. Here we find embryologists approaching the problems of development from an entirely different standpoint. The egg is looked upon as a physical mechanism of extreme complexity, and an attempt is made to analyze the changes into their component elements, i. e., to determine the factors that lead to, and complete each successive event of the development. But here also we find that however probable the conclusions may be, there always remains a doubt that can not be removed, so long as the method of observation alone is used. The results may have the appearance of greater exactness, since the conclusions are stated in physical and chemical terms, but we have here no new method, but only a different set of problems to study.

It may appear that the embryologist is doomed to disappointment in whatever direction he turns, but there remain to be examined the results of another method by means of which the study of embryology may be approached. I refer to the method of experiment.

The physicist and the chemist have found, that the only method by means of which they can establish their principles, i. e., the sequence of connected phenomena, is by means of experimentation. An intelligent understanding of the purpose of the experiment must, of course, precede the attempt to alter the conditions in such a way as to determine which changes in the complex are dependent on each other, and which are only concomitant. The more complete our information gained by observation, the more intelligible will be the application of the experimental method. In fact, without preceding knowledge of what is to be done, the experiment can have very little value, unless, as is sometimes the case, the right method is hit upon by a happy chance, and the connection of events made evident.

The application of experiment to the solution of the problems of embryology is not new, but within the last fifteen years a much larger number of workers have made use of this method, and with some success considering the difficulties to be overcome.

One of the earliest applications of the experimental method was made in order to determine whether the active element in the seminal fluid, the spermatozoön, brings about the development of the egg, or whether the spermatozoa are simply parasitic protozoön-forms found living in the male fluid. To determine this point Spallanzani separated the spermatozoa from the fluid by means of a filter, and the fluid was then applied to unfertilized eggs. It was found that the eggs did not develop, while the same fluid with the spermatozoa left in it, and also the spermatozoa left on the filter, when applied to the eggs started the development. We now know from direct observation, that a spermatozoön enters the egg; but at the time, when Spallanzani made his experiment, this could hardly have been ascertained by observation alone with the methods and instruments then in use.

The more recent study of embryology by means of experi-

mental methods dates from Pflüger's paper, in 1883, on the effect of gravity on the segmentation of the frog's egg. Pflüger's attention was drawn to the fact that the first and second divisions of the frog's egg are vertical, and the third division, that lies at right angles to the two preceding, is horizontal. The experiments were made in order to determine if there exists any connection between the factors that bring about the division of the egg and the force of gravity. The normal egg rotates freely in its membranes after fertilization, and, owing to a difference in weight, or specific gravity, of its black and its white hemispheres it turns so that its black pole is upwards, and its white pole is downwards. The first division of the egg starts at the upper pole, and the plane of division passes vertically downwards through the egg, dividing it into halves. The second plane of division also begins in each half in the upper part, and passes vertically downwards, so that the egg is divided into four similar quadrants. The third planes are horizontal, and in each quadrant the plane separates an upper, somewhat smaller, dark part or cell from a lower, larger, white part. The subsequent divisions are not so regular, but in general it may be said, that in the black hemisphere the division takes place more rapidly than in the lower hemisphere.

In order to find out if gravity plays any part in determining the position of the division planes, Pflüger tried turning eggs into different positions before the division or cleavage of the egg takes place. The egg is prevented from rotating in its membranes by adding only enough water to allow the spermatozoön to enter, but not enough to allow the egg membrane to swell as much as under normal circumstances. In consequence the membrane presses upon the egg and keeps it in any position in which the egg may be placed.

If gravity, which always acts vertically, determines the position of the division planes, then in an egg with the dark hemisphere

turned to one side, we should expect the first division to appear at the top of the egg, and pass vertically downwards, and not to appear first at the black pole as in the normal egg, since this now lies at one side.

It was found that the first plane of division begins at the highest point of the egg irrespective of what part of the egg may lie in this position. In fact, the first plane of division may, in some cases, divide the egg so that the black hemisphere may be separated from the white hemisphere. The second plane of division is also vertical, and the third horizontal. The results seem to show that gravity is a factor that determines in some way the position of the planes of division of this egg.

A normal embryo develops from these eggs; and Pflüger concluded that since the egg may be divided in all sorts of ways in response to gravity according to the position in which it is placed, and since a normal embryo is produced that bears no relation to the planes of division, there exists no causal relation between the cleavage and the position of the embryo on the egg. This conclusion, it will be noticed, is not necessarily connected with Pflüger's conclusion that gravity determines the position of the planes of division; and while, as we shall now see, this latter conclusion is, in a sense, erroneous, the other conclusion, in regard to the relation between the embryo and the cleavage planes, is one that has been confirmed and has an important bearing on later work.

A year later Roux put Pflüger's conclusion, in regard to the action of gravity, to a new test. Fertilized eggs of the frog were placed in boxes, and these were put on a rotating wheel. When a certain velocity of rotation was given to the wheel, the eggs that were free to move in their membranes turned so that the heavier white hemisphere was directed outwards, i. e., they orientated themselves with regard to the centrifugal force instead of the force of gravity. The eggs as they rotate will, of

course, at every moment, change their relation to the direction in which gravity acts on them. The planes of division appear in these eggs as in the normal, and consequently can not be the result of the direction in which gravity has acted on them, because the relation has not been constant. Since, however, the new planes of division now stand in the same relation to the centrifugal force of the revolving wheel, as do the normal planes to the force of gravity, it may be supposed that the former has been substituted for the latter. It was, therefore, necessary to change the experiment in such a way, that neither the force of gravity, nor the centrifugal force acted in the same direction on all the eggs. It is possible to do this by making the wheel rotate more slowly, so that the eggs do not orientate themselves in regard to the centrifugal force, but lie in whatever position they may happen to be placed. Under these circumstances, Roux found that each egg divides in the same way that the normal eggs divide; and since some eggs have one position and some another in regard to the centrifugal force, and since the eggs have changed at every moment their relation to gravity, it is clear that the egg may divide without regard to either of these factors. It is highly probable, in consequence, that there is only a coincidence, and not a causal connection between the position of the cleavage planes in the normal egg, and the action of gravity on the egg.

Pflüger's conclusion from his experiment is shown to be erroneous; but Roux's results leave unexplained the facts that Pflüger observed in the obliquely-turned eggs.

A series of experiments made in the same year, 1884, by Born, give us an insight into the meaning of the cleavage of the eggs in Pflüger's experiment. Born examined by means of sections the interior of eggs placed and held in oblique positions, and found that a slow rotation of the contents takes place in such a way, that the lighter parts of the egg move toward the top of

the egg, and the heavier parts move to the bottom. In consequence of this change, the contents of the egg assume the same position in regard to the vertical as the normal eggs. Division of the egg begins at the highest point, i. e., at the top of the egg, and passes thence through the egg, dividing it into equal portions, therefore passing downwards through the heavier part of the egg. Gravity has acted on the egg in such a way, that a rearrangement of the contents takes place, so that the lighter parts move upward, and the heavier parts downward. The relation of these parts to each other in the egg determines the cleavage; and gravity is not connected with the division, except in so far as it brings about a rearrangement of the lighter and heavier parts of the egg before division.

Pflüger had overlooked this change in the interior of the egg, because the outermost layers of the egg do not take part in the rotation, and the egg appears to retain the position in which it is turned; but this, as Born's results show, is only true for the harder, denser, outer layer of the egg.

The second conclusion that Pflüger drew from his experiment is not upset by the disproof of his conclusion in regard to the action of gravity; for even admitting a rearrangement, more or less complete, of the contents of the egg, still the position of the embryo in the obliquely-turned egg was found to bear no relation at all to the position in which the cleavage planes appeared. This part of Pflüger's results has not as yet been questioned. The conclusion is all the more striking, since, as first determined by Newport, in 1851, the first plane of division of the frog's egg corresponds, in many cases, in position to the later median plane of the embryo.

This series of experiments is, I think, instructive as giving an insight into the experimental method. We see that wrong conclusions may be drawn from experiments, but we also see that

the method gives us a means of testing the conclusions themselves; in fact, the scientific value of the conclusions will be, in general, directly in proportion to the extent to which they may be tested. We can not overestimate, I think, the practical side of the experimental method, in the sense that the results attained will have just so much value as scientific data, as they can be further tested by the experimental method itself.

We may next examine another series of experiments, that have profoundly influenced our ideas in regard to the problems of development. Omitting all details, and giving only the general outlines of the experiments, the facts are as follows: Roux, after independently discovering that the plane of first division of the egg of the frog corresponds, in many cases, to the median plane of the embryo, tried the experiment of injuring one of the first two cells, in order to see if the uninjured half would produce only half of the normal embryo. By piercing one of the first two cells with a hot needle he delayed in some cases, prevented in others, the development of the injured half. The uninjured part continued, nevertheless, to develop, and produced exactly half of the normal embryo. Roux thought his experiment showed that, at the first division, the egg divides qualitatively into the material to form the right and the left halves of the embryo. He concluded, also, that at each further division of the cells of the egg, there is a qualitative separation of the material. This conclusion it will be observed is diametrically the opposite of that which Pflüger formulated. Roux's conception is the basis of Weismann's speculation as to the method of embryonic development, and it will be profitable to give a somewhat detailed examination and criticism of this view.

We may consider Roux's idea of a progressive differentiation during development, either from the narrower standpoint from which his adversaries have attempted to treat it, or if we like from a

broader point of view. In the former respect, more recent results have not confirmed Roux's conclusion; but in the latter, his idea of a progressive differentiation has, to a certain extent, been established.

Roux attempted to show how the qualitative division of the egg is brought about. He pointed out that the spindle that is formed during each cell-division, and by means of which the separation of the chromatin takes place, is an instrument by which we can conceive a qualitative distribution of the chromatic material to be accomplished. The chromatin is looked upon as a substance bearing the essential qualities of the individual, as is demonstrated by the inheritance of the characters of the father through the nucleus of the spermatozoön. This same idea of a qualitative separation at each division of the qualities of the chromatin is, as I have said, also the central idea of Weismann's hypothesis.

Subsequent discoveries have not been favorable to this conception. I submit the following facts that substantiate, I think, this claim. In certain eggs, in which the protoplasmic products of division are very unequal and where the division of the protoplasm is into different kinds, and the products of the division produce entirely different parts of the embryo, we find the chromosomes always dividing into exactly equal parts. Whenever the chromatin divides, the same kind of separation takes place, so that, so far as the evidence of direct observation goes, it is opposed to Roux's view. In the second place, as we shall see more fully a little later, each of the first two, or first four cells, in several forms, if separated, gives rise to an entire embryo and not to a half or to a fourth; and further in another experiment, in which a part of the protoplasm is removed from the egg, but the entire nucleus left, the egg develops into a part of an embryo, and not into a whole one.

Before we discuss further this view of a progressive differenti-

ation during development, some further experiments that have a direct bearing on the question must be described.

The results of Roux's experiment on the frog's egg were complicated by the presence of the injured half of the egg that remained in contact with the developing half, and further, because the cell pierced by the hot needle is not killed, but only injured so that its development is either delayed or prevented. If, however, the process of development is not due to a qualitative division at the first cleavage, but is the outcome of a mutual interaction of the cells, then we can imagine that the injured cell might still react on the developing cell as though it also were developing. In fact, Roux himself thought that, in the later stages of development, an interaction of the parts must take place; and later Hertwig extended this view to the whole development. Although it has not been possible to carry out the experiment of completely separating the first two cells in the frog's egg, yet in a related form, salamandra, Herlitzka has recently succeeded in completely separating the first two cells, and has found that each develops into an entire embryo. Even in the frog's egg it is possible to show that each of the first two cells may give rise to a whole embryo by a somewhat different experiment. If the egg is inverted, after the first cleavage is completed, and held in this position, two embryos fused together in different ways are formed. It appears that this result is due to gravity bringing about a partial rearrangement of the contents of each cell before the next division-planes come in; and in consequence of this rearrangement, each cell continues to develop as a single egg.

A few years after Roux's experiment, Chabry carried out a similar one on the egg of an ascidian. By means of an ingenious apparatus he succeeded in controlling the egg, so that he could pierce with a needle any particular cell of the embryo. The

injured cell was, in many cases, completely removed. His results were not, however, as definitely stated as could be desired; and later experiments by Driesch, have shown that Chabry missed the real meaning of the experiment.

The next experiments in chronological order are those of Driesch, and his results occupy an important place in the history of modern experimental embryology.

By means of a method first used by the Hertwigs, Driesch succeeded in shaking apart the first two, (and also the first four) cells of the egg of the sea-urchin. Each cell continues to develop at first as though still in contact with its fellow, i. e., as a part, and not as the whole egg develops, and a hollow hemispherical embryo, open at one side, is produced by each isolated cell. So far the development has been strictly a half-development. Later, however, the open side closes in, and a hollow sphere is formed, which continues to develop as does the normal egg, and each produces at last a perfect embryo, but of half or fourth the normal size. The result is, in some respects, the opposite of that obtained by Roux, since a whole, and not a part of an embryo, develops from each isolated cell. It must not be forgotten, however, that at first the development is strictly a half-development, and that only in the later stages a whole embryo is formed.

The experiment of separating the first-formed cells was soon carried out with the eggs of a number of other animals belonging to widely different groups, and while in some of these the results were similar to those with the sea-urchin, yet, in other eggs, different results have been obtained.

Wilson found, for instance, that, after separating the cells of the egg of amphioxus, the isolated cell sometimes divides as a part, and sometimes as the whole egg divides. The isolated piece responds, as it were, in some cases at once, and develops as a whole. There can be little question, I think, in the

light of recent work, that very slight changes may determine whether an isolated cell may continue to develop as a whole or as a part, the result depending, perhaps, on the more or less symmetrical arrangement that the contents of the cell have assumed when division takes place. It would be a mistake, I think, to infer that there is any fundamental difference between an egg that divides as a part, and one that divides as a whole.

It has been found that if the first two cells of the egg of the ctenophore are separated each not only continues to divide as a part, but forms only a part of an embryo, that corresponds in many respects to a half-embryo. The egg of the ctenophore represents one end of the series, and that of amphioxus the other end.

It may seem that the half-development of the isolated cell of the ctenophore egg is a proof of the correctness of Roux's hypothesis; but there are some further facts to be considered that put the whole problem in an entirely new light.

If, after the egg of the ctenophore has been fertilized, a piece be cut off from one side of the egg, one of the pieces, that, of course, that contains the nucleus, may continue to develop; but it produces in most cases not a whole embryo, but a part of an embryo, resembling the embryo of this animal obtained from the first two, or the first four cells. In this case the entire nucleus is present, and yet only a part of an embryo develops. The result is obviously directly connected with a loss of the protoplasm that represents the missing part.

In the light of this experiment it is evident that we can not interpret the partial development of the isolated cell as due to a qualitative division of the nucleus, but rather, as in the latter experiment, to the absence of half of the protoplasm. The most obvious interpretation of the experiment with the ctenophore egg is that the structure, or arrangement of the protoplasm, is of such a sort that it determines the mode of division of the egg, and even

the formation of the embryo. In this egg there is no rearranging of the material of the egg after separation of the cells, or removal of a part of the protoplasm of the unsegmented egg. In other words, the form of the embryo is the outcome of the protoplasmic structure of the egg.

This view, held especially by Driesch and by myself, is diametrically opposed to the Roux-Weismann conception of the process of development.

It may be asked, how it is possible to harmonize this view with the well known fact that the spermatozoön brings into the egg very little, if any, protoplasm, but mainly a nucleus, and that, nevertheless, the embryo in its later stages may show characteristics of the father as well as of the mother. The contradiction is, I believe, only apparent.

At the present time, evidence is accumulating that shows that we can not regard the chromatin as something isolated in the cell; but that there is a free interchange of material between the nucleus and the surrounding protoplasm. As a result, the protoplasm will be, in time, affected by the kind of chromatic substance contained in each cell, and when the specific characters of the cells develop the influence of the chromatin, introduced into the egg by the spermatozoön, will appear. I may, therefore, state my conclusion in this way: that in so far as the protoplasm has, at any stage been affected by substances received from the nucleus, it will show this influence when it produces structures that are of the sort to be affected by the change in the protoplasm. Whether a half, or a whole embryo develops from an isolated cell, or from a piece of an egg also depends on the structure of the protoplasm; but at this time the structure may have nothing whatsoever to do with the kind of material in the nucleus since this material is not of the sort to affect the half or the whole arrangement of the protoplasm.

This problem of the influence of the chromatin on the protoplasm, is a question that is at the present moment, receiving much attention on the part of cytologists and embryologists. Boveri carried out an interesting experiment by means of which the influence of the nucleus on the protoplasm could be tested. It is possible, as we have seen, to break the unfertilized eggs of the sea-urchin into pieces by shaking them violently in a small tube. The pieces subsequently round up, and while some will contain the female nucleus of the egg, others will be entirely without a nucleus. Boveri tried to fertilize the egg-fragments of one species with the spermatozoa of another species in order to see what influence the nucleus of the spermatozoön would have on pieces without an egg-nucleus. He found that from the fragments two kinds of embryos developed; most of them were midway in structure between the maternal and paternal types, and these he thought must have come from the nucleated fragments; a few were in structure exactly like the paternal type of embryo, and these Boveri thought came from the pieces without a nucleus that had been entered by a single spermatozoön. If this result could be completely established it would be most important, since it would show that the nucleus has greater influence on the character of the embryo, than has the protoplasm. This conclusion would not be, however, fatal to the view of development of Driesch and myself, since it must be freely admitted that the nucleus may influence the protoplasm. According to our view a part of an embryo would develop from a piece of the fertilized egg of the ctenophore or from an isolated blastomere regardless of whether the egg had been fertilized by a spermatozoön of the same, or of another species. The structure of the protoplasm that determines the form of the cleavage and even the form of the embryo, so far as it is incomplete, may not have anything to do with the chromatin present in the nucleus, or at least not

be influenced by it in this regard during development. If the egg had been fertilized by a spermatozoön of another species, and then the first two cells separated, we should expect, on our view, to get two half-embryos, but each half would be midway between the maternal and the paternal types, because there has been an interchange between the nucleus and the protoplasm of that kind of material that has an influence on the structures of the later stages of the embryo.

The value of the evidence on which Boveri based his conclusion has, however, been questioned. It has been shown that when entire eggs of the same species used in Boveri's experiment are fertilized by spermatozoa of the other species, while the majority of the embryos are intermediate in character, a small percentage are more or less nearly the same as the paternal type. Hence, as the same is probably also true for the nucleated pieces of the egg, Boveri's evidence is undermined, although the possibility that the non-nucleated pieces develop, as Boveri claims, still remains.

There is another series of experiments of a different kind that have given us important information in regard to the factors at work during development. Pflüger first showed that by compressing the frog's egg between two parallel glass plates the sequence of divisions, i. e., the position of the division planes is altered; the first three planes, at least, being at right angles to the compressing plates. The egg that has divided in this way will give rise to a normal embryo, either under pressure, or if the plates have been removed after the divisions have taken place. We have here two problems to consider; namely, the influence that the pressure has in determining the change in the division of the egg; and secondly, the production of a normal embryo from an egg that has divided differently from the normal.

The same experiment has been made on other eggs with the

same result. The effect of compression is particularly well shown on the sea-urchin's egg, in which a flat plate consisting of a single layer of sixty-four cells has been produced. These flat plates, if released from pressure, also give rise to normal embryos. Even in eggs in which a definite relation is known to exist between the first formed cells and definite parts of the embryo, a normal embryo may be formed after the cleavage has been altered by compressing the egg, as Wilson has shown for the egg of Nereis. Curiously enough it has been found, by Ziegler, that the division of the egg of the ctenophore under pressure is very nearly the same as the normal cleavage, and this result is all the more interesting in connection with the experiments described above on the method of development of pieces of this egg. The result bears out the assumption that the protoplasmic structure of the ctenophore egg is so fixed, i. e., so difficult to readjust that external factors have less influence on it than on other kinds of eggs.

Several hypotheses have been advanced to account for the change in the division of eggs under pressure, but none of them are entirely satisfactory. Pflüger first directed attention to the influence that pressure might have in determining the position of the nuclear spindle, and, in consequence, on the position of the plane of division that comes in at right angles to the long axis of the spindle. Pflüger thought that the spindle takes the position of least resistance, which would be parallel to the compressing plates. It has been objected to this view, that if the egg is a semi-fluid body enclosed in a more solid envelope the pressure would be equalized in all directions. Hertwig attempted later to give a formula along the same lines to cover the results. According to Hertwig's view the nucleus tends to assume the protoplasmic centres of the egg or of the cell; and the spindle, when it forms, assumes the position of the greatest protoplasmic mass. This hypothesis, while it seems to cover a number of observed cases,

fails entirely in other cases. For instance, an egg compressed vertically in its plane of symmetry does not have a longer protoplasmic region except in so far as the egg is broader in a horizontal plane than in a vertical plane, but there are an infinite number of possible positions for the spindle in this horizontal plane, and some other factor must determine which one is to be chosen. The hypothesis will also not explain several cases in which the position of the spindle is not determined by the direction of the greatest protoplasmic mass, but by some other internal factor. It may be stated, therefore, that as yet we do not know what factors influence the position of the spindle in the dividing egg.

The results of these experiments have an important bearing on the relation of the embryo to the egg, for, as has been stated, the embryo has a different relation to the position of the planes of cleavage in the compressed and in the normal egg. Of greater interest is the fact, that, owing to the change in the sequence of the divisions, the parts of the egg receive different nuclei (at least if the numerical sequence of the division of the nucleus is considered) than in the normal egg. If the series of division of the chromatin is into qualitatively different parts, as believed by Roux and by Weismann, we should expect to develop from the egg that has divided under pressure either a monstrous form, with all the parts combined in unusual ways, or else nothing at all, because of the dislocation of the elements. On the contrary, a normal embryo develops, which seems to demonstrate the incorrectness of the hypothesis of the qualitative division of the chromatin. If, on the other hand, we look upon the division of the chromatin as a division into like parts at every division, then it is a matter of indifference what particular product of the division is sent to each part. Further, if the planes of division of the egg also bear no necessary relation to the formation of the organs of the embryo, then, again, we

find no difficulties for our view of development. Finally, if we look upon the entire protoplasm as the factor that determines the origin of the organs, we find this arrangement is, in all essential respects, the same whether the egg is compressed or not.

The results of the experiments with isolated cells show that the protoplasm of some eggs has even the power of rearranging itself into a new whole after a part has been removed, and this result we find paralleled by the power possessed by many embryos, and by a number of adult animals, of forming a new animal out of any piece of the old one.

Roux has tried to meet the difficulty, that his view encounters in connection with these experiments, by assuming that the nucleus also responds to the change in its environment and proceeds to divide under compression in such a way that each part of the egg receives the same constituents of the nucleus as that part receives in the normal egg. The hypothesis has no observed facts to support it, and is obviously an attempt to cover a vital weakness in the primary assumption of a qualitative division. It assumes on the part of the nucleus a power to meet a difficulty that would puzzle the highest intelligence to work out; for, if at each division of the nucleus there is an unravelling of the qualitatives of the individual, it is certainly taking great liberties with the hypothesis to assume that in response to a slight compression of the protoplasm and a corresponding change in the nuclear spindle, that an entirely new sequence of qualitative divisions could take place at once, and of exactly the right kind for each part of the egg to get its proper nuclear constituents, i. e., that the qualitatively different nuclei shall stand in the same spacial relation to each other as in the normal egg.

The conclusions that Driesch and Hertwig have drawn from these experiments are, I think, the legitimate ones. According to their interpretation the early divisions of the nucleus are always

into equal parts, and the daughter nuclei are all qualitatively alike, and like the nucleus from which they have come. The results of the division of the egg will be to distribute chromatic material to all regions. The changes that later take place in the different regions are not due to the kind of chromatin that each part has received, but to another factor, and this other factor is, according to the view of Driesch and myself, the structure of the protoplasm.

There is a further question of some importance that is not included in the foregoing statements. Do the nuclei always remain alike or do they also suffer a change as development proceeds? There is a good deal of evidence to show that, in most animals, only in a certain region of the embryo are all the qualities of the original egg retained i. e., in the reproductive cells; and that the other cells of the body have lost, to a varying degree, the totipotence of the early cells. It is difficult to decide whether this change is only due to a change in the protoplasm, or whether the nuclei have also undergone a progressive change. If there is, as I have supposed, an active interchange between the protoplasm and the nucleus, we can readily conceive that the nucleus may in time lose certain of its earlier possibilities. At present the evidence is insufficient to establish this view; but it seems not improbable that it may turn out to be correct.

There remains, finally, to be discussed a point that has scarcely been touched upon in the preceding pages, although it is connected with some of the phenomena already discussed. The egg is, before fertilization, in nearly all cases radially symmetrical around a principal axis, yet in many cases a bilateral embryo develops from such an egg. The question arises as to what factor changes a radical structure into a bilateral one. If an external factor, what can it be? If an internal one, it must be present from the beginning; and if so, the appearance of a symmetrical

structure in the egg is deceptive. The results of observations have shown that in certain eggs, having a definite bilateral form, as the eggs of certain insects, the embryo appears orientated in a definite position in the egg. It may seem that the bilateral form of the egg itself determines in the later stages the bilateral form of the embryo. It must not be overlooked, however, that the bilateral form of the unsegmented egg is itself the result of an internal bilateral structure, since the egg has made its own form during its growth, and its form has not been determined by the surrounding conditions. This internal factor may, or may not, be connected with the factors that produce the bilateral embryo.

Roux was the first to attempt to show that an external factor might determine the median plane of the egg of the frog. By means of a fine pipette, filled with fluid containing spermatozoa, he set free the latter at a definite point near the surface of the egg. This point was noted, and it was found that subsequently the first plane of division passed through the point of fertilization. Since, in the frog's egg, the first plane of division corresponds more or less nearly with the median plane of the embryo, the factor that determines the position of the embryo in the egg is the point of entrance of the spermatozoön. We may then look upon this egg as in reality radially symmetrical around a principal axis, and an external factor determines the position of the embryo. The bilateral embryo does not exist preformed in the egg, in any sense, for there are present in the egg before fertilization an infinite number of possible planes, any one of which might become the median plane of the embryo. Around the principal axis any part, at a given level, may form the same part of the embryo, and the fate of each part is determined by the selection of the median plane of the embryo. The results show, in a most striking way, that we can not project, in imagination, the embryo upon the unfertilized egg for the latter contains the pos-

sibility of an infinite number of embryos, and an external factor decides which one shall be chosen.

The first plane of division, as we have seen, passes through the point of entrance of the spermatozoön, and also through the centre of the egg and the highest point of the black hemisphere. It may seem strange that the point at which the spermatozoön enters the egg should play so important a rôle in the subsequent development; but we have at present other observations that indicate that it is not the point of penetration itself, but that this is connected accidentally, as it were, with other factors, and the latter determine the position of the first plane of division. The male nucleus on entering the egg passes towards the centre of the egg and towards the female nucleus; the two nuclei come in contact, flatten against each other, and subsequently fuse into a single sphere. We have some direct evidence, in other eggs, to show that the chromatin material of the two nuclei, although it fuses, does not mix. When the spindle develops it assumes a position midway between the male and female halves of the nucleus; or, in other words, if the nuclear halves do not change their position after fusion the spindle will lie at right angles to the path of entrance of the spermatozoön in a horizontal plane. After the separation of the chromosomes the first plane of division of the egg which passes through the middle of the spindle, will be, therefore, also in the path of entrance of the male nucleus, and cut through the point at which the spermatozoön entered the egg. This analysis of the problem, for which I must assume the responsibility, gives us a deeper insight into the relation between the first plane of cleavage and the fertilization of the egg. We also see that the correspondence between the point of entrance of the spermatozoön and the median plane of the embryo need not exactly correspond, (in fact Roux and others have found that the correspondence

is not always exact) and still the explanation that I have offered will hold, for should the male nucleus not enter in a straight line, or should the segmentation nucleus shift its position the plane of division will correspondingly change, but the factors that determine the division plane remain always the same.

In the preceding pages I have attempted to give a general idea of the problems of development that are engaging the attention of working embryologists at the present time. My main purpose has been to show what the results of the historical method have been as contrasted with those of the experimental method. I do not see the necessity or the advantage of weighing the relative merits of each method, for so long as we admit that there are problems of both kinds, we may rest assured that certain problems will appeal to certain minds, and other problems to other minds; and if I choose to think that the experimental method is the most profitable method, I am willing to admit that the study of embryology might be undertaken even from an artistic point of view,—and with success!

The main theme of dicussion of embryologists during the last century, and the present century as well, has been the relative importance of preformation versus epigenesis. By preformation it was formerly meant that the embryo exists already formed in the egg in miniature; and that its development is simply a question of enlargement, as the child becomes a man. This view was held by nearly all the eminent embryologists of the last century. In opposition to this conception Wolff attempted to show that the chick does not exist preformed in the egg, but arises out of two flat plates or layers that roll themselves up into the form of the chick; that the development is from a simple condition to a more complex one, that it is a process of building up, or epigenesis.

In the present century the idea of epigenetic development, as

enunciated by Wolff, has been entirely accepted; but the idea of preformation has assumed a new form in the hands of modern thinkers. It is assumed by certain modern embryologists, or whom Weismann may be taken as an example, that there exist in the chromatin of the egg certain bodies that correspond to each character of the individual, and that the process of development consists in the sorting out of these bodies to the different parts of the egg. There they act on their respective regions and cause them to develop into their respective organs. Thus the parts of the embryo exist in the egg, and may have even a definite relation to each other, although not necessarily in any way corresponding to the relation of the parts of the later embryo to each other. In other words, not a miniature chick, but the elements of the chick are preformed. This is modern preformation.

On the other hand, other embryologists claim that it is erroneous to suppose that the egg contains any such preformed bodies; but that the development is the result of a series of changes becoming more and more involved as the stages are passed through. The egg may be looked upon as a very simple structure as compared with the chick, but its qualities are of such a sort that once started into activity they produce by their interaction a complicated result. This is modern epigenesis.

A number of embryologists maintain that both views contain an element of truth; that certain very definite qualities must be present in the egg, for, unless this were the case, the end-result would not be as definite as we find it. To them it seems inconceivable that all the eggs of an individual could produce such similar end-results, unless an extremely complicated structure of some sort were present at the beginning.

It seems to me that we have inherited, in this question of preformation versus epigenesis, one of those delicately balanced notions of the school-men; and that we might go on to the end

of time discussing the relative merits of the two *ideas*. The results of the study of embryology from the experimental standpoint show us, I think, where we can profitably direct our energies; and it is my personal opinion, that the sooner we drop the questions of preformation versus epigenesis the better for the future study of embryology. We have seen that at each stage in the development of the egg a large number of problems present themselves for our further examination. It is the separation of the factors of development into their components by means of an experimental test, that demands our attention. Hertwig has set up the paradox that each stage in the development is the cause of the next stage, and maintains that in a description of the series of stages through which the embryo passes we find the causal explanation of the development. If Hertwig had stated that at each stage there exist in the embryo the factors or causes for the following stage he would, I think, have been nearer the mark. Our object is to separate these factors into their components; in other words, there is no one problem of development, but a vast number of connected problems.